A Summary of Current Program 7/1/67 *-*

and Preliminary Report of Progress

for 7/1/66 to 6/30/67

ENTOMOLOGY RESEARCH DIVISION

of the

AGRICULTURAL RESEARCH SERVICE

UNITED STATES DEPARTMENT OF AGRICULTURE

and related work of the

STATE AGRICULTURAL EXPERIMENT STATIONS

Section B

This progress report is primarily a tool for use of scientists and administrators in program coordination, development, and evaluation; and for use of advisory committees in program review and development of recommendations for future research programs.

The summaries of progress on USDA and cooperative research include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed, will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to members of Department staff, advisory committee members, and others having a special interest in the development of public agricultural research programs.

This report also includes a list of publications reporting results of USDA and cooperative research issued between July 1, 1966, and June 30, 1967. Current agricultural research findings are also published in the monthly USDA publication, Agricultural Research. This progress report was compiled in the Entomology Research Division, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Md.

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TABLE OF CONTENTS

		Page
Section B		
Area No. 13	Livestock Insects and Other Arthropods	175
Area No. 14	Insects Affecting Man, Households, and Industrial Establishments	213
Area No. 15	Bees and Other Pollinating Insects	236
Area No. 16	Analysis, Synthesis, Formulation, and Evaluation of Insect Control Chemicals	251
Area No. 17	Identification of Insects and Related Arthropods	267
Area No. 18	Foreign Exploration, Introduction, and Evaluation of Biological Control Agents	282
Area No. 19	Insect Pathology	292
Area No. 20	Insect Physiology and Mode of Action of Insecticides and Their Metabolites ...	306
Area No. 21	Fundamentals of Insect Sterility	322
Line Project	Check List	329

AREA NO. 13. LIVESTOCK INSECTS AND OTHER ARTHROPODS

Problem. Insects and other related arthropods attack all classes of livestock and poultry causing estimated annual losses of $877,850,000. Losses are attributed to direct attack of arthropods, causing losses in weight gains and milk and egg production, and losses in the value of livestock products such as meat, hides, and wool. Additional losses result from livestock and poultry diseases spread by arthropod vectors. A large variety of insects and other arthropods affect livestock including mosquitoes and biting gnats, house flies, horse flies and deer flies, ticks and keds, the face fly, the stable fly, the horn fly, cattle grubs and other bots, lice, mites, and fleeceworms. Practical but not adequate control methods for many of these livestock pests have been developed, but no satisfactory methods of protecting livestock and poultry from mosquitoes, biting gnats, horse flies, deer flies, and stable flies have been found. Development by insects of resistance to control chemicals is a continuing threat to current effective chemical methods of control. The occurrence of insecticide residues in meat and animal products restricts the usefulness of some chemical control methods. Continued basic and applied research is needed to develop new, safer, more effective chemical control agents and methods of using them as well as other methods, such as management practices, sterilization, attractants, and biological control into highly effective integrated means of control or eradication. Research is also needed to study the role of insects in the spread of diseases of livestock and poultry.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing, long-term program involving basic and applied research on the biology and control of insects and related arthropods which affect the health and productivity of all classes of livestock. The total Federal scientific effort devoted to research on livestock insects is 28.5 scientific man-years and includes studies on: (1) beef, horse and swine insects; (2) dairy cattle insects; (3) sheep and goat insects, and (4) poultry insects. Research is conducted on: (A) basic biology, physiology, and nutrition; (B) conventional insecticide control methods; (C) insect parasites, predators, and pathogens; (D) insect sterility and other new approaches to control; (E) insecticide residue determinations; (F) attractants; and (G) insect vectors of animal diseases. The following tabulation indicates the distribution of scientific man-years between commodity groups (1-4) and types of research (A-G).

	A	B	C	D	E	F	G	TOTALS
1	2.5	3.3	1.1	2.0	0.7	0.4	0.6	10.6
2	1.5	3.3	1.2	2.5	0.7	0.4	0.6	10.2
3	1.0	1.0	0.1	---	---	0.3	1.0	3.4
4	0.5	1.2	1.1	1.0	0.1	0.4	---	4.3
Totals	5.5	8.8	3.5	5.5	1.5	1.5	2.2	28.5*

* Plus 1.5 man-years program leadership

Federal support in research grants, contracts, and cooperative agreements provides for 4.6 scientific man-years. Commodity distribution is 1.9 to beef insects; 1.4 to dairy insects; 0.9 to poultry insects, and 0.4 to sheep and goat insects. Research area distribution is 2.1 to basic biology, physiology, and nutrition; 0.8 to conventional insecticide control methods; 0.8 to insect parasites, predators, and pathogens; 0.5 to insect sterility and other new approaches to control; and 0.5 to attractants and repellents. These extramural research projects are located at the following institutions: University of California at Berkeley, University of California at Davis, University of Georgia, University of Kentucky, University of Southwestern Louisiana, McNeese State College, Louisiana State University and A & M College, Mississippi Agricultural Experiment Station, Mississippi State University, State College, New Mexico State University, Oklahoma State University Experiment Station, University of Utah, Virginia Polytechnic Institute, University of Wyoming, Instituto Nacional de Investigaciones Agricolas (Mexico), and University of Nebraska Agricultural Experiment Station.

Additional research is conducted under P.L. 480 grants. A13-ENT-3, "Investigations on the biology of dung beetles in Korea and their role in the prevention of fly breeding in dung," at the Department of Agricultural Biology, College of Agriculture, Seoul National University, Suwon, Korea; A10-ENT-12, "Laboratory study of tick repellents and acaricides," at the Veterinary Institute, Beit Dagan, Israel; S9-ENT-7, "Investigations on natural enemies of ants," at el Centro de Investigaciones de Fruticultura, Horticultura y Vitivinicultura, Montivideo, Uruguay; and F4-ENT-6, "Studies on the control of house flies and mosquitoes by means of chemosterilants in Egypt," at the University of Cairo, Cairo, Egypt.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 39.4 professional man-years is devoted to this area of research.

PROGRESS USDA AND COOPERATIVE PROGRAM

A. Basic Biology, Physiology, and Nutrition

1. Mosquitoes, sand flies, and gnats. At Gainesville, Fla., studies are being conducted to obtain biological information needed to accurately evaluate the potentialities of chemosterilants against mosquitoes. Data obtained recently indicate females of Culex pipiens quinquefasciatus may mate more than once if confined with large numbers of males. However, sperm from second matings is utilized only if the matings occur within a few hours of each other. Normal males have been found capable of mating with 2 females per night. Males sterilized with apholate can mate with only about 1 per night.

Additional information has been collected on the action of P^{32} in mosquitoes. Tests have shown that Anopheles quadrimaculatus larvae pick up more radioactivity when exposed to P^{32} in distilled water than infusion water. Increasing the dosage and exposure time increased the activity in the insects but also increased the mortality that occurred. There is a direct relationship between P^{32} activity in the male and the amount found in females inseminated by these males.

At Lake Charles, La., studies were continued on the biology of salt marsh and rice field mosquitoes. Tests were continued with screened and unscreened enclosures. Initially, it appeared that most of the eggs had hatched by the end of the first year. By then, the hatch of eggs was smaller in the screened enclosures than in the unscreened ones. However, during the second year some hatching of Aedes sollicitans, A. taeniorhynchus (2 salt-marsh mosquitoes) and Psorophora confinnis (major rice-field species) continued, even on the 28th flooding. A comparison of eggs laid in the spring and fall suggests that warm weather species A. taeniorhynchus and P. confinnis, especially the latter, lay some egg batches in the late fall. Most of the fall eggs are in a deep diapause state and are less hatchable than eggs laid in the spring. Apparently A. sollicitans eggs do not diapause in the Gulf Coast region; no hatching differences were noted between spring and fall eggs with this species. Ninety-three water samples were analyzed from habitats of salt-marsh Aedes spp. and P. confinnis. Salt concentrations that produced confinnis averaged one-third to one-half as many as those that bred A. sollicitans and A. taeniorhynchus. No pH correlation was noted. In 24 oviposition preference tests, P. confinnis showed a preference for low salinity compared with A. sollicitans and A. taeniorhynchus, but all three species showed definite preferences for increasing concentrations of chloride salts. In further tests, the salt-marsh species sollicitans and taeniorhynchus showed no preferences among chloride salts, but sollicitans avoided sodium carbonate and sodium bicarbonate and these salts were detrimental to sollicitans eggs. Sollicitans eggs hatched in water with a sodium chloride concentration as high as 6.6%. A. taeniorhynchus eggs hatched in water containing as much as 2.0% chloride and P. confinnis eggs hatched at 1.0% chloride.

At Corvallis, Oreg., research continued on the biology of mosquitoes. Aedes dorsalis and A. melanimon are difficult to separate taxonomically. The

same situation occurs with Aedes excrucians and A. aloponotum. It is possible that differences within these complexes may be physiological and due to the habitat. An attempt is being made to establish a colony of A. dorsalis and field sites have been chosen for ecological studies of larval requirements or preferences of dorsalis and melanimon. A seasonal decline in concentration of alkali metals in the water samples from these sites was noted, but further studies will be needed before any conclusions can be drawn. In further biology observations, it was noted that larvae of Mansonia perturbans either develop unevenly or the egg-laying season extends over a considerable period. Aedes increpitus is a fall species; the earliest recorded brood occurred in October, before any killing frost. Thus, exposure to freezing is apparently not a hatching requirement. The commonest species of mosquito breeding this year in log ponds was Culex peus, with lesser breeding of C. p. pipiens, C. tarsalis, and Culiseta incidens in most localities. However, Culiseta incidens was the dominant species at one rural locality and the proportion of pipiens was higher in the urbanized community of Eugene, Oreg., than in more rural settings. A factor in undefatted liver is toxic to larvae of Culex tarsalis. Extracting fat solubles yields a product suitable for larval food.

Captures of insects feeding on a horse showed black flies biting all day long, but highest catches in the late afternoon. Culicoides gnats were most numerous in the morning. Anopheles freeborni, Culiseta inornata, and Aedes dorsalis fed during the day. Studies were initiated to determine which Oregon species of Culicoides gnats feed on poultry.

Research has continued under two contracts at the University of Southwestern Louisiana and McNeese State College. Mosquito breeding was light around Lafayette, Louisiana, particularly in the last half of 1966, compared to previous years. The same situation occurred around Lake Charles, La. Several heavy broods of larval mosquitoes failed to produce comparable populations of adult mosquitoes, suggesting some biological control agent was at work attacking the larvae.

Research was initiated under a grant to Virginia Polytechnic Institute at Blacksburg, Va., on the biology of Culicoides gnats. Initial studies indicate that man is more attractive than small mammals or birds and that the standard New Jersey light trap (used for mosquitoes) and an animal-baited trap, designed by the Principal investigator and a fellow scientist, were the best of several designs tested for trapping these gnats.

Research was initiated under a grant at Louisiana State University, Baton Rouge on the determination of host animals of mosquitoes in certain areas of Louisiana. Blood was collected from species of birds, mammals, amphibian and reptiles. Antisera were prepared and tested for specificity for all animals collected. Preliminary results indicated that of 8 mosquito species collected in 4 parishes the majority had obtained blood meals from beef, a few from horses or swine, and occasionally from rabbits and deer. A few had fed on two hosts.

2. House flies (All Livestock). At Corvallis, Oreg., research continued on the genetics of house flies. The inheritance of resistance was determined by crossing according to standard genetic procedures. Resistance to organophosphates and carbamates was linked to the 5th chromosome semidominant genes. Chlorinated hydrocarbon resistance was associated with these genes and with 2nd chromosome recessive genes. Resistance to the cyclodiene group of hydrocarbons was associated with a gene or genes other than the 2nd and 5th chromosome genes. Noninsecticidal carbamates were found in some instances (nearly 200 tested) to synergize carbamate and organophosphate insecticides. In one instance, resistance was reduced from 300-fold to 5-fold or less by the combination. The results support the hypothesis that different alleles of the same major gene confer resistance to carbamates and organophosphates in the house fly.

3. Stable Flies (Cattle and Horses). In Oregon, colonies of stable flies were maintained on varying numbers of blood meals per week. One colony which has existed for six generations on three blood meals a week now oviposits as well as a colony fed five blood meals a week. Another colony was maintained with two and five blood meals a week given to alternate generations.

In Texas, in cooperation with the Agricultural Engineering Research Division, a system for recording stable fly flight activity was constructed. An electrometer probe placed in a fly cage and connected to a recorder continuously records the flight of stable flies under varying conditions.

4. Horn Flies (Cattle). The percentage of female horn flies mated at different ages in a laboratory test conducted in Texas were: 1 day old, 0%; 2 days old, 12%; 3 days old, 63%; 4 days old, 83%; and 5 days old, 89%. Other tests indicate that one male horn fly will inseminate 1 to 6 females over a 7-day period.

An insect bitometer was constructed at the Kerrville, Texas, laboratory in cooperation with the Agricultural Engineering Research Division. The bitometer was used to study the *in vitro* feeding activity of horn flies. The results of tests conducted under continuous light indicate that horn flies do not tend to feed at a certain time during the day.

In Texas, an improved artificial horn fly larval medium was developed. The medium contains 264 g ground sugarcane pulp, 12 g fish meal (livestock feed grade), 48 g whole wheat flour, and 6 g sodium bicarbonate. The number and size of horn fly pupae produced in this medium equal or exceed the production from cow manure.

Studies were initiated on the biology and ecology of horn flies in cooperation with Mississippi State University, State College, Miss. Differences were found in the sex ratios of field collected horn flies. For example, during March the percentage of males was 56%, while for April through June

it decreased--the range was 34%-47%. Phenomenon of diapause is being studied in environmental chambers. Larval development was extended using 12 hour light with constant temperatures.

5. Horse Flies, Deer Flies, and Snipe Flies (All livestock except poultry). In Mississippi, collection of horse flies from a bait animal indicated the feeding activity is strongly influenced by outdoor environmental conditions. A peak of activity was observed from 8:30 to 9:30 a.m. Secondary peaks tend to occur during periods of highest light intensity. There seemed to be a negative correlation with relative humidity and a positive correlation with dew point. Feeding-landing ratios for eight species varied from 59% for the Tabanus atratus to 5% for T. subsimilus, the most abundant species.

At Fresno, California, in cooperation with the University of California, research was initiated on horse flies, deer flies, and snipe flies. Snipe flies are biting flies roughly resembling deer flies in their manner of attacking man and livestock. Malaise traps baited with carbon dioxide caught numbers of snipe flies that correlated well with numbers collected from tame deer. However, horse flies and deer flies responded better to the traps than to the deer. A trap consisting of rotating nylon nets caught many more horse and deer flies than the Malaise trap. There is a tendency (most pronounced on young deer) for snipe flies to bite or to be allowed to bite on the face rather than the ears.

Research under a grant to the University of Utah was initiated on the biology and control of tabanids in marshlands along the eastern shore of the Great Sale Lake. Six species of tabanid flies were collected in the study area. Chrysops discalis the most abundant and pestiferous species deposited eggs only on vegetation or other objects over water. A total of 11 different plant species were used for oviposition. The adults emerged only from moist soil. The counting of pupal cases on the surface of the soil was a suitable method of determining the number of emerging adults in a given area.

Research is continuing under a grant to the University of Wyoming on the biology of tabanids. A small scale mark-recapture study of Tabanus reinwardtii and Chrysops fulvaster indicated that both sexes of C. fulvaster but only males of T. reinwardtii remained close to flowing creeks whereas marked females of T. reinwardtii were collected on horses 3 miles away. Males of both species are active only during 7 to 10 a.m., while females remain active from 6 a.m. until sunset. Mating of both species was observed many times and motion pictures were made. All mating occurred between 7:30 and 11:00 a.m. at temperatures between 75 and 88° F.

Research under a grant to Louisiana State University was initiated on the determination of host animals of tabanids in certain areas of Louisiana. Blood was collected from species of birds, mammals, amphibian and reptiles. Antisera were prepared and tested for specificity for all animals collected. Preliminary results indicated that of the 7 species of tabanids collected from 6 parishes, the majority had fed on beef, a few on horses, and one each on a rabbit and a deer.

6. Face Flies (Cattle and Horses). At Corvallis, Oreg., a survey was made for the presence of face flies. This survey conducted in the central, eastern, and northeastern sections of the State failed to show the presence of face flies in 1966.

Prior to July 15, 1966, face fly populations were lower in eastern Nebraska than in previous years during the same period. Counts were three flies or less per animal. Numbers increased to 10 or more per animal soon after mid-July and remained high for the duration of the season. Flies began to congregate at overwintering sites during the week of September 19. Some had entered buildings and clustered on September 20, 1966.

Tests in Nebraska showed that face fly larvae migration prior to pupation ranged from 0.5 to 30 feet from the cow dung in which they developed. The distance that the larvae migrated depended on environmental conditions and ground cover. Aleochara tristis larvae, a parasite of face flies, traveled 12 to 14 feet to parasitize face fly pupae.

7. Little House Fly (Poultry). At Corvallis, Oreg., an orange-eyed strain of the little house fly, F. canicularis, was established from progeny of a mutant female observed in the laboratory's stock Fannia colony. Later, a white-eyed strain was isolated from the orange-eyed strain.

Tests on the inheritance of the characters have demonstrated that the orange-eyed condition is inherited as a simple recessive. The white-eyed condition is a modifier of dominance such that flies homozygous for orange eyes and heterozygous for white eyes are yellow-eyed, while flies homozygous for both conditions are white-eyed. Flies homozygous for white eyes but not homozygous for orange eyes are wild type.

The mutants are being used in experiments on the sexual biology of Fannia, and should prove useful in studies on insecticide resistance.

8. Screw-worm (All livestock except poultry). Sterile flies released at the rate of 1000 flies per square mile per week were not effective in reversing a rising trend in screw-worm populations in a 5496 square-mile area in Sinaloa, Mexico. There were indications from the ratios of sterile to fertile egg masses collected from pens of wounded animals that the effects of the released males decreased as a function of distance within 2 to 3 miles from the line of release.

The seasonal cycle of screw-worm activity in Sinaloa followed much the same pattern previously noted on the east coast of Mexico, i.e., peak populations in the spring and fall and relatively low populations coinciding with dry weather in summer.

In cooperation with the Department of Agriculture of Mexico, trapping studies in a deep, narrow canyon in western Mexico indicate that sterile flies will

disperse into canyons of this type after having been released over mountainous terrain at altitudes up to 12,500 feet.

Studies were continued on the possible changes in longevity, fecundity, and behavioral characteristics of screw-worm flies as a result of adaptation to artificial rearing and holding procedures. The mean longevity of wound-reared females of a recently introduced Mexico strain was 28.5 days as compared to 21 days for females of this strain reared on artificial medium. There was no statistically significant difference in longevity attributable to sex. Flies of the Mexico strain lived longer than flies of the older Florida strain. Females of both the above strains reared from artificial medium were less fecund than females reared from wounds, and the average eggs per egg mass was more variable in the former group. There was no evidence that selection for adaptation to artificial medium had affected fecundity in either strain. Wound-reared Florida strain flies previously reared on artificial medium for over 150 generations laid an average of 352 eggs per egg mass compared to an average of 344 for Mexico strain females from a stock that had been reared exclusively on wounds.

Tests are being continued to determine the minimum size range for male screw-worm flies without impairment of physical ability to mate with wild type females. Males from prepupae weighing less than 50 mg mated 51% of the available populations of wild type females compared to 79% for males from prepupae weighing 60 to 70 mg.

The time required for screw-worm larvae to obtain the capacity to pupate varied from 52 hr on artificial media to 94 hr on wounds. The critical larval weight for successful pupation ranged from 26 to 30 mg. Pre- and postcritical growth curves are being used to evaluate the suitability of various artificial media.

A new device for permitting free oviposition by mated female screw-worm flies is yielding data on potential egg production from field populations of flies. The rate of egg mass depositions as a function of local population densities is needed for the interpretation of the results of field studies.

The longevity of laboratory-reared screw-worm flies was determined by recording the daily mortality of bisexual and unisexual populations and of solitary flies. Males and females in unisexual populations outlived those in bisexual populations, an indication that an adverse effect on longevity was produced by the interaction of the sexes. Solitary males and females outlived males and females in unisexual populations, an indication that an adverse effect on longevity was produced by grouping the flies. Females outlived males when the two sexes were segregated or when the flies were solitary, but this difference was largely masked when the sexes were mixed.

When screw-worm flies were released and observed for 10 days in a laboratory room, survival of females and fertility were improved when the tile floor was covered with cheesecloth. The cheesecloth probably provided a more

secure footing for the flies than tile so that more matings were successfully terminated than were interrupted prior to completion.

Although egg production was similar for 5-, 10-, and 15-day-old female screw-worm flies, the fertility of eggs laid by 5-day-old females was greater than that of 10- and 15-day old females, which was equal.

When 7-day-old females from bisexual populations were weighed and allowed to oviposit individually, the relationship between body weight and the number of eggs laid was linear. For each additional milligram of body weight, the number of eggs laid was increased by an average of 4.5 eggs.

Tests on hydroponic media for screw-worm larvae were conducted in cooperation with the Animal Health Division at Mission, Texas. Dehydrated food-stuffs were compounded into hydroponic type media for rearing larvae of screw-worm. Mixtures containing whole blood, calf suckle, and whole egg supported larval growth and development equal to regular media of fresh meat and blood.

9. Cattle Grubs (Cattle). At Kerrville, Texas, it was found that during the pupal period of Hypoderma bovis, weight and specific gravity decreased rapidly in days 0 to 4, and after that the decrease was gradual. Volume of the pupae decreased slightly during the 22.6-day period for females and 21.5 day period for males. Oxygen consumption followed the characteristic "U"-shaped curve, with lowest levels at 6 to 8 days after larvae had egressed from animals' backs.

At Corvallis, Oreg., Hypoderma bovis pupae that were held in the laboratory emerged approximately 15 days earlier than field-reared specimens. Survival of the puparia reared in the laboratory was nearly 10% greater than those reared in the field. Sex ratio for both field and laboratory specimens was 50%.

A study of cattle grub morphology was conducted at the Kerrville, Texas, laboratory in cooperation with Purdue University. Studies of the three larval instars of Hypoderma lineatum indicate that there is a complete digestive tract in all instars. It consists of a pharynx, esophagus, ventriculus, intestine, and rectum. Malphighian tubules were identified in all larval stages. The respiratory system consits of functional paired anterior and posterior spiracles with connecting tracheae and tracheoles.

10. Ticks (Cattle). A laboratory colony of Dermacentor albipictus has been established at Kerrville, Texas. All parasitic stages were raised on bovine in a stanchion. Over 2600 engorged females were recovered from one calf. Engorged females weighed an average of 0.42 to .5 g; preoviposition period is 10.8 days; oviposition period is 23.2 days; average egg mass contains 3600-4200 eggs.

The ticks, Boophilus annulatus and B. microplus were cross-mated under controlled conditions and the females of both species produced fertile

eggs. Brother x sister crosses of F_1 hybrids indicated that most of these progeny are sterile; back crosses of F_1 hybrids to pure strain annulatus and microplus indicated there is much more sterility in hybrid males than females. Some fertile hybrids were produced in 2 of 16 crosses of microplus ♂ x annulatus ♀.

11. Mites (Poultry). Research continued on the biology of Neoschongastia americana, a mite, which is a serious pest of turkeys, under a grant at the University of Georgia. The mites showed no preference as to either sex or age of turkeys. Mite population cycle peaks occurred at two-week intervals. A lesion is started by one mite and others are apparently attracted by secretions from the initial wound, which are located on the inside and outside of the thighs and only rarely on the breast. Lesions caused by mites feeding reached a point beyond which the leasion was incapable of supporting mites.

B. Insecticidal and Sanitation Control

1. Mosquitoes. At Gainesville, Fla., the search for new and safer insecticides for mosquito control was continued. The most effective materials screened against larvae of Anopheles quadrimaculatus were experimental compounds ENT-27386 and ENT-27444 which gave 100% control at 0.01 ppm. Five other commercial compounds killed all of the larvae at 0.025 ppm.

Comparative tests conducted with the standard larvicide technique have shown Dursban and Abate to be two of the most efficient larvicides that have ever been developed for the control of Aedes taeniorhynchus. They have been 2 to 3 times better than parathion, the most effective compound tested previously and considerably more effective than fenthion, malathion, naled, or DDT. In wind-tunnel tests against adult mosquitoes, six insecticides were evaluated that appear to be as effective as the malathion standard. Eleven synergists have also been found that increased the toxicity of Bay 39007 at least 2 times.

The development of ultra-low-volume sprays has received major attention at the Gainesville laboratory. Of the materials tested so far naled, fenthion, and Bay 39007 have proven to be more effective than malathion. A comparison of ultra-low-volume and conventional sprays showed naled to be about equally effective in both types of application. Fenthion, however, was about 20% less effective as an ultra-low-volume spray than as a conventional spray 6 hours after application, but both methods of application caused about the same reduction after 24 and 48 hours. Malathion was slightly less effective as an ultra-low-volume spray than as a conventional spray regardless of the time interval after treatment.

Bay 39007 has been found more effective than malathion as an aerial fog against natural infestations of adult salt-marsh mosquitoes, Aedes taeniorhynchus and A. sollicitans in Florida.

Additional research has been conducted with a nonthermal aerosol generator developed to disperse insecticides from the ground. Fenthion, naled, Bay 39007, Bay 41831, Schering 34615, Dursban, and Shell SD-8211 and an experimental compound, ENT-27334, have been highly toxic to adult females of Aedes taeniorhynchus, Anopheles quadrimaculatus, and Culex pipiens quinquefasciatus in tests with this equipment. When thermal and nonthermal aerosol applications of DDT and malathion were compared, the two pieces of equipment produced similar mortalities. DDT at 16% was ineffective against Aedes taeniorhynchus and Culex pipiens quinquefasciatus but produced 89 to 100% mortalities of Anopheles quadrimaculatus. Malathion at 4% produced 64 to 99% kill for 300 feet against all 3 species.

Larval selection with DDT for nine generations increased the resistance in a strain of Anopheles quadrimaculatus, that was already known to possess some resistance, to a level that was 2,333 times above that of the laboratory colony and 152 times above that of the parent strain. Adults of this strain exposed on panels treated with insecticides showed resistance to DDT >10,000 times higher than the regular strain of Anopheles quadrimaculatus but were nonresistant to malathion.

Studies are still in progress to develop new insecticides that can be used as residual sprays where Anopheles mosquitoes have become resistant to DDT and dieldrin. In laboratory tests 6 chemicals have been sufficiently effective to justify testing in buildings naturally infested with mosquitoes.

Research was continued at Corvallis, Oreg., on the evaluation of insecticide resistance and the development of insecticides. Resistance to DDT was demonstrated in Culex tarsalis at Oakridge, Oreg., 10 years ago. Although DDT has been used little or not at all for mosquito control there since, the resistance persists. It could be reasoned that some selective factor probably is present that favors the resistant strain. Most probably this is continued use of various larvicides; thus, pressure must be supposed on the DDT-resistance gene. Abate, Dursban, and fenthion are all highly effective against mosquitoes resistant to DDT and malathion in a laboratory strain of Culex p. pipiens. Larvae of Mansonia perturbans showed high tolerance to malathion and DDT and a surprising amount of tolerance to fenthion, with some evident even to Dursban and Abate. These appear to be natural tolerances.

Abate and Dursban are two of the most promising mosquito larvicides. Last year's tests gave little residual larvicidal action at the low dosages tested; slightly higher dosages (0.06 lb Abate/acre and 0.035 lb Dursban/acre) gave averages of 13 days and 18 days protection, respectively for the two materials.

Eighty-four compounds were evaluated as repellents and toxicants for wild Aedes dorsalis mosquitoes in spot tests on cattle. Three of the compounds had shown promise in 1965; they again showed good repellency for several days.

About 3 dozen potential systemic insecticides were tested. Most interesting were the results obtained with tests in which 6 insecticides were given orally to cattle. All caused some mortality of Aedes aegypti feeding on the treated cattle. The best were trichlorfon and famphur, which gave 98 and 100% kill, respectively, at the highest dosage tested. Another interesting finding in these tests was that stable flies were more susceptible than mosquitoes. Several compounds ineffective against mosquitoes also killed stable flies.

Research was continued under contract with the University of California to evaluate promising insecticides for mosquito control in low-volume applications. Promising results were obtained with low-volume applications of fenthion and Dursban. Mosquito control in pastures was usually 100%, but results were less effective in dense plantings of rice fields until the airplane nozzles were pointed forward, reducing droplet size and apparently increasing penetration.

Research was conducted under cooperative agreement with the University of Florida to study the genetic basis of resistance to chemosterilant by a mosquito. Chemosterilant dosages are being explored as a prelude to genetic tests.

2. House flies. At Gainesville, Fla., the search for new insecticides effective in controlling house flies was continued. In laboratory tests, 5 experimental materials were more effective than the ronnel standard against insecticide susceptible and resistant house flies. Residual tests against house flies in Florida dairy barns were conducted with 9 insecticides. Dimethoate and fenthion were the most effective, but they produced more than 50% control for only 3 to 14 days.

Research was continued at Corvallis, Oreg., on the development of insecticides and the evaluation of insecticide resistance. The resistance spectra of 8 strains of house flies were measured in tests with 13 insecticides (organophosphates, carbamates, chlorinated hydrocarbons, and pyrethrins). Some strains were resistant only to a limited number of insecticides, others were resistant to most materials tested.

In other studies with a parathion-resistant strain of house flies, increased tolerance occurred to phosphates containing O-methyl esters, but lesser tolerance to phosphates containing di-n-propyl or diisopropyl esters. Exposure of malathion-resistant house flies to malathion synergized with DEF (S,S,S-tribuytl phosphorotrithioate) resulted in increased resistance. However, the increase in resistance was only 5-fold in 29 generations, suggesting that combinations of organophosphates and synergists or use of O,O-dialkyl phosphates show promise for control of flies resistant to standard phosphate insecticides.

Commercial 20% dichlorvos resin strips were found to be highly effective on larvae of flies breeding in sewage pits at a slaughter house. Mortality of

larvae was complete within 5 days and some reduction in fly breeding continued another week, even after removal of the dichlorvos resin strips.

At Beltsville, Md., investigations of physical methods for fly control were continued in cooperation with the Animal Husbandry and Agricultural Engineering Research Divisions. Research on the effectiveness of farmstead sanitation confirmed previous findings that significant house fly reductions (about 33%) can be achieved by sanitary measures on a single farm when unsanitary farms are close as 1/2 mile. Flies dispersed primarily upwind and to "dirty"areas with many potential breeding sites, rather than to "clean" areas.

Research was conducted under cooperative agreement with the University of Florida to study house fly insecticide resistance. Continuous exposure was found to be the simplest method of determining resistance in house flies.

3. Screw-worm (All livestock exceptpoultry). Of 15 compounds tested at Mission, Texas, as larvicides against newly hatched screw-worm larvae at three concentrations, 10, 1.0, and 0.1 ppm, in the larval medium, 8 were highly effective, killing all of the larvae at 1.0 ppm. Four of the compounds screened were effective at 0.1 ppm.

4. Stable Flies (Cattle and Horses). In Texas 211 new compounds were screened in spot-tests on cattle for repellency and toxicity against the stable fly. Seven of these compounds were Class IV toxicants at concentrations of 0.5% or lower. One compound was a Class IV feeding repellent, due to its rapid knockdown action.

Large cage tests were conducted to evaluate two chemicals for the control of stable flies. Conventional 2-qt spray applications of 0.25% Dursban and 0.25% of an experimental compound, ENT-27405, were effective 3 to 10 days against stable flies.

In Oregon, six systemic insecticides were administered orally to cattle to determine their relative toxicity to stable flies and mosquitoes. Stable flies were more susceptible to the insecticides than mosquitoes, except for famphur where little difference was noted. Fenthion and famphur were more toxic to insects at lower dosages than the other materials tested. The longest lasting material was an experimental compound, ENT-22377, which caused mortality of the insects for two or more weeks.

Research continued under contract with the Florida State Board of Health at Panama Beach, Fla., on control of dog flies (stable flies). Naled continues to look promising as an adulticide, but DDT is the most effective larvicide applied to beach grasses where these pests breed. Bay-39007 appears to be about as effective as DDT for this use. DDT is relatively ineffective against adult flies. Bay-39007 is about as effective as DDT in the beach grass piles.

5. Horn Flies (Cattle). In Texas, large cage tests were conducted to evaluate two chemicals for the control of horn flies. Conventional 2-qt spray applications of 0.25% Dursban and 0.25% of an experimental compound, ENT-27405, were effective for 15 to 18 days.

Two herds of cattle at Camp Stanley, Texas, were sprayed with 2 qts/head of 0.25% Shell SD-8447 for horn fly control. The compound provided less than 13 days control.

An automatic sprayer for ultra-low-volume (ULV) application of insecticides (1-ml per application) to cattle has been developed in cooperation with the Agricultural Engineering Research Division. Laboratory studies indicated that the following concentrations of insecticides would be required to maintain horn fly control on animals utilizing the ULV sprayer on a daily basis (1-ml applications twice daily): carbaryl 1.0%; Ciodrin 0.5%; Compound 4072 0.25% or 0.1%; coumaphos 0.25%; malathion 1.0%; and methoxychlor 1.0%. Milk residue studies with 2% and 5% malathion resulted in no residues in the milk. Ronnel at 5% concentration resulted in a maximum residue of 0.014 ppm and at 10% a maximum residue of 0.037 ppm. Field tests demonstrated that ULV sprayers will control horn flies on dairy herds, using 0.5% Ciodrin spray.

At Kerrville, Texas, tests indicate that horn flies resistant to ronnel may have increased tolerance for coumaphos, trichlorfos, Shell SD-8447, dioxathion, malathion, and bromophos.

Two insecticides were tested against the larvae of a ronnel resistant strain of horn flies and a ronnel susceptible strain of horn flies. The larvae of the ronnel resistant strain showed a high tolerance for both ronnel and Dowco 177.

In Texas, bioassays were run to determine the toxicity to horn fly larvae of manure from cattle fed three insecticides at varying rates for 10 days. All the materials reduced larval survival but only famphur at 5 mg/kg daily gave 100% mortality.

A component in the water insoluble (pH 7.0) fraction of Bakthane, a commercial product of Bacillus thuringiensis, was responsible for the activity against horn fly larvae in feces from cattle treated orally. Larvicidal activity was found after oral treatments with separate spore and crystal fractions; however, minute amorphic particles from the parent material remained with both spores and crystals after separation.

In Mississippi, field tests were conducted for the control of horn flies, using five methods of application and six insecticides. Control was obtained with the insecticides applied in dust bags, back rubbers, as water based sprays, as oil-based sprays, and as pour-ons. Most treatments afforded excellent horn fly control.

Research was initiated under cooperative agreement with the Delta Branch Experiment Station, at Stoneville, to determine the efficacy of insect

control on cattle as related to beef production. Preliminary data from the initial tests of this long term study indicate that the techniques being tried had a detrimental effect on weight gains.

6. Cattle Grubs (Cattle). In Oregon, Imidan as an 0.375% spray proved highly effective in reducing cattle grub numbers. However, Imidan provided poor control when sprayed at 0.25%. Imidan at 5 g/hd as a pour-on was highly effective. Two other compounds tested showed systemic activity but even at the highest dosages tested, activity was lower than desired.

Research was continued in Texas and Oregon to develop more effective insecticides for the control of cattle grubs and other bots affecting livestock. At the Kerrville, Texas, laboratory 55 new compounds were screened for systemic action by giving them orally and subcutaneously at several dosages to guinea pigs infested with larvae of Cochliomyia macellaria and Phormia regina. Stable flies and nymphal lone star ticks were also allowed to feed upon the treated animals. Twenty-four materials exhibited systemic activity in one or both types of administration.

In Texas, 43 systemics were tested for effectiveness against cattle grubs (H. lineatum and H. bovis) on both native and imported Wyoming cattle. Each treatment group consisted of small numbers (3 to 4) of these government-owned cattle. Materials considered effective were those providing 90% or greater control of cattle grubs. In these tests, three were effective as sprays alone, one as a spray and in the feed, one as a pour-on, one in the feed materials, and two in oral capsules. In large scale tests on cooperator-owned cattle, 74 and 79% control of grubs was obtained with 0.05 and 0.1% sprays of fenthion, respectively, and a 2% pour-on of fenthion applied at 1/2 oz/cwt provided 76% grub control.

7. Nose bots (Sheep and goats). Basic studies on the nature and significance of the mode of action of systemic insecticides against sheep nose bots when administered to sheep by different routes was conducted under a grant to the University of Kentucky. Thirty-two lambs were artifically infested each with 25 1st instar larvae of the sheep nose bot. Six compounds were administered in capsule form at one mg/kg per day for a 10-day period. Four days later the animals were sacrificed and examined. Fenthion and famphur killed all the 1st three instars of larvae of the sheep bot.

8. Ticks (Cattle and Horses). During the early summer of 1966, two tests were conducted at Camp Stanley, Texas, with 2-year-old Hereford heifers naturally infested with lone star ticks to evaluate the efficacy of insecticidal sprays in controlling these ticks. Bromophos-ethyl, Bay 39007, Imidan, Dursban, and Banol were applied to cattle at various concentrations in different formulations (1 gal/hd) and the effectiveness in controlling lone star ticks was compared to a standard 0.5% toxaphene spray. All treatments, except bromophos-ethyl (0.25%) and Dursban (0.01%), were as effective as toxaphene (0.5%) in controlling ticks at one day posttreatment. At one

week posttreatment, only 0.1% Bay-39007 and 0.01% Dursban were as effective as 0.5% toxaphene. In general, at two weeks posttreatment, numbers of ticks on treated cattle were equal to numbers on untreated cattle.

Personnel at the Kerrville, Texas, laboratory continued research on the evaluation of insecticides for the control of Boophilus ticks on cattle. Of 87 insecticides used in screening tests to determine activity against Boophilus annulatus and/or Boophilus microplus, only 12 were placed in Class IV (effective at 0.01%). Materials considered effective were those that reduced egg laying or egg hatch by at least 90%.

Field tests were also continued in Mexico to evaluate the effectiveness of promising insecticides as sprays and dips for the control of Boophilus ticks on cattle. Sprays of 0.03% coumaphos EC (as Asuntol); 0.01% and 0.03% coumaphos EC (as Co-ral); 0.5% Mobil MC-A-600; 0.25% Banol; 0.1% Bay-39007; 0.15% Ciodrin; 0.06% coumaphos WP; 0.5% menazon; 0.05% diazinon; 0.075% dioxathion; 0.03% coumaphos WP; and 0.5% carbaryl gave 96 to 99.95% control of reproduction. Dipping trials with Dursban, Shell SD-8447, and carbaryl provided high or complete control of reproduction. Dursban that had been allowed to age for nine months in the vat, still provided greater than 99% control of tick reproduction. On the other hand, Shell SD-8447 at six weeks failed to control ticks due to the formulation combining with dirt, debris, etc., and settling to the bottom of the vat.

In Texas, 16 formulations of 11 insecticides were applied to the ears of 35 cattle for control of the ear tick, Otobius megnini. Only the following afforded ≥94% control of nymphs at 1 month posttreatment: Coumaphos 4% pour-on, 5% dust, and 0.25% in aerosol with 0.5% trichlorfon; and Dursban 0.1% emulsion.

A technique has been developed at the Kerrville, Texas, laboratory in which cattle are used as hosts for the tropical horse tick, Anocentor nitens. A large number of engorged females have been raised for laboratory insecticide tests. Testing procedures similar to those used to determine the effectiveness of insecticides for the prevention of reproduction of Boophilus ticks have been used to obtain similar data for A. nitens. Of 65 compounds tested for ixodocidal activity against A. nitens, 23 were effective at a concentration of 0.01%.

The Kerrville laboratory has also established a colony of the winter tick, Dermacentor albipictus, by raising all parasitic stages on bovines in a stanchion. Engorged female D. albipictus were dipped in 30 insecticides by techniques developed for Boophilus screening tests. Compounds placed in Class IV (effective at 0.01%) were: Lindane, isobenzan, Shell SD-8448, and experimental compounds ENT-24967 and ENT-25869.

Research on insecticidal methods of controlling ticks was continued at Gainesville, Fla. In a field test Bay-39007 was highly effective as a toxicant against a natural infestation of fowl ticks, Argas persicus in a

commercial poultry house. Reductions of 99 to 100% were obtained for at least 193 days with a spray containing 2.5% Bay-39007, for 109 days with 1% Bay-39007, and 81 days with 0.5% Bay-39007.

Research was continued under PL-480 with the Ministry of Agriculture at Beit Dagan, Israel on tick repellents and acaricides. The ortho isomer of deet (N,N-diethyl-ortho-toluamide) was toxic and repellent to Hyalomma excavatum and repellent to Rhipicephalus secundus ticks. Ortho-deet was about 10 times more repellent than dimethyl phthalate. Dimethyl phthalate showed no toxicity (acaricidal effects) to ticks at the dosages tested. Preliminary tests indicate oil of Citronella is about one-tenth as acaricidal and benzyl benzoate is about one-fifth as acaricidal as ortho-deet to ticks.

9. Lice (Poultry). In Kerrville, Texas, louse populations on leghorn hens were eliminated by dusting with 4 g/bird of a commercial preparation of Bacillus thuringiensis containing spores, crystals, and toxins. Four to five weeks were required for complete control with a single dusting; multiple treatments reduced the control time to about three weeks.

Thirty-one selected toxicants were evaluated for control of poultry lice by 40-ml direct spray to the bird treatments of 0.25% or less concentrations. Twenty-nine of the toxicants eliminated all motile forms of lice on the birds in seven days or less. Ciodrin was outstanding and gave complete control at a concentration of 0.025%.

Direct application in spray tests of an experimental compound ENT-27395 eliminated Northern fowl mites from hens at a concentration of 0.1%. Litter treatments with Shell SD-8447 at a rate of 1 lb/100 ft^2 eliminated poultry lice, but not at 0.5 lb/100 ft^2.

C. Biological Control

1. Mosquitoes. At Gainesville, Fla., research on pathogens of mosquitoes has been continued. Larvae of Anopheles quadrimaculatus and Aedes aegypti exposed to Nosema spores became heavily infected. The A. quadrimaculatus were more susceptible than A. aegypti to the disease. Primary sites of infection are the fat body, gut, and malphigian tubules. Stempellia magna has been found infecting larvae and adults of Culex restuans. A new species of Stempellia has been found in Culex pilosus. Both species are transmitted transovarially and cause high mortality in larvae. Attempts to transmit them per os to other mosquitoes have failed so far. Thelohania legeri has been found in two Anopheles spp. in Florida and its life history is being studied in the laboratory. Stages of the pathogen have been found in late instar larvae, pupae, and female adults but have not been observed in first instar larvae. Thelohania opacita was found in Culex territans.

Coelomomyces fungi have been found in the larvae and adults of Anopheles crucians. In one test Coelomomyces fed to early first instar larvae of

A. quadrimaculatus resulted in heavy infections in several specimens. A fungus of the genus Rubettella was found in the larvae of two Culex spp.

Two types of Mosquito Irridescent Virus have been obtained from larvae of Aedes taeniorhynchus. One caused the larvae to be an irridescent pinkish color, and the other produced a bluish-green color. Both types have been transmitted to the first and second instars of Aedes taeniorhynchus in the laboratory. These infections produced approximately 10% mortality. The adipose tissue appears to be the primary site of infection, and as the disease progresses the fat body cells become hypertrophied and eventually rupture, releasing numerous viral bodies into the haemocoele. The viruses have been purified sufficiently to begin electron microscope studies.

At Corvallis, Oreg., research continued on pathogens of mosquitoes. A fermentation product of Bacillus thuringiensis was toxic to larvae of Culex tarsalis, with an LC-90 of about 40 ppm during the larval development period. Toxicity was expressed as failure to survive and emerge as adults. A major problem in use of pathogens is the lack of methods to infect a field population of mosquitoes with the pathogen. Excystation of Thelophania spores can be induced with exposure to gaseous osmic acid, or exposure to oxygen followed by desiccation over calcium chloride. Osmic acid was found to be toxic to Culiseta incidens larvae. Oxygenation was more effective in spores frozen and held at 4° C. for about 8 months than with spores kept at room temperature for the same period.

At Lake Charles, La., research on mosquito pathogens as potential biological control agents continues to receive increasing emphasis. Microsporidian (and other protozoan), bacterial, fungal, viral, and nematode parasites continue to be found in various species of mosquitoes. Colonies of the following mosquitoes have been developed and are being maintained (the note in parentheses indicates a regular strain and one harboring the type of pathogen listed): Aedes aegypti (virus), A. taeniorhynchus (virus), A. tormentor (Thelohania, a microsporidian), A. triseriatus (Thelohania), Culex salinarius (Thelohania), C. pipiens quinquefasciatus (Plistophora, another Microsporidian), Culiseta inornata (Plistophora), and Psorophora ferox.

Protozoan pathogens have been found in 17 species of mosquitoes and include 3 genera of microsporidia and 1 genus of flagellate. One, the Plistophora in Culiseta inornata, has been maintained in the laboratory through 10 generations of mosquitoes. It is transmitted transovarially.

A bacterium, probably a species of Vibrio, was found in three species of mosquitoes and was transmitted to larvae of two other species of mosquitoes in food.

Fungal infections were found in 9 species of mosquitoes. Some kinds were readily transmitted to other mosquitoes in the laboratory.

At least two species of virus infecting mosquitoes have been found, and 4 species of mosquitoes have been found to be hosts. One virus has been maintained in the laboratory through 62 successive generations. This virus is readily transmitted to another mosquito species and back to its parent species. However, the other virus is readily transmitted to its host species, but to only one percent of a second species of mosquito.

Nematodes were observed in 6 mosquito species. Over 90% of some Aedes sollicitans populations were found to be infected in nature. The nematode in this mosquito is believed to be Agamomermis culicis, reported early in this century but not fully described. It causes the death of many female mosquitoes, and those that survive are unable to lay eggs in normal numbers.

The fact that all of these mosquito pathogens have been found in the field may cast some doubt on their potential usefulness at this time in controlling mosquitoes naturally present in Gulf Coast marshes. However, they may be a principal factor holding mosquito populations in check in "light mosquito years." Further research may show how to increase the efficiency of the pathogens to prevent "heavy mosquito years."

Research was continued under a contract with the California State Department of Health at Fresno to study pathogens affecting mosquitoes. A polyhedral virus was found in a field population of Culex tarsalis. The fungus Beauveria bassiana kills mosquitoes in laboratory tests, but results were disappointing in simulated field tests. Attempts are being made to increase its virulence, or improve the method of infecting mosquitoes. Mosquito infections with protozoa (Stempellia and Plistophora) are also being studied.

Research was initiated under a grant to the University of California at Riverside on the use of introduced annual fishes as a means of mosquito control. Early studies with the fish Cynolebias bellottii, C. wolterstorffi, and Nothobranchius guentheri indicate C. bellottii is the most promising, at least for California conditions. Eggs are laid even at temperatures near freezing and withstand 10 weeks burial in mud. Development to maturity requires about three weeks after hatching of the eggs.

Research has continued under contract with McNeese State College at Lake Charles, La., on predators and parasites of mosquito larvae. Collections of arthropods were made and identified in the hope of finding good control agents. Corixid bugs and Cyclops (crustacea) did not appear in laboratory tests to be predaceous, though this is reported in the literature. Three types of native minnows consumed large numbers of mosquito larvae (up to a hundred or more per fish per day) and these studies will be continued.

2. House Fly. Research also continued in Korea with dung beetles under PL-480. Several species have been found that are easy to rear, and some progress has been made in learning which species most efficiently renders manure unsuitable for fly breeding. A grant to the University of California is showing excellent progress on the effects of manure removal schedules on

population dynamics of immature stages of six species of filth flies and on their predators. Biweekly sampling continued all year to determine annual cycles of abundance for the fauna of the manure. Wood shavings under the manure increased the abundance of predaceous beetles but not the mite or maggot population with the possible exception of Stomoxys. In the laboratory the mite Macrocheles was found to prey on eggs of Stomoxys, Ophyra, Fannia, and Muscina.

3. Imported Fire Ant. Under PL-480 support, research was continued on parasites of the imported fire ant in Uruguay. Studies with the parasitic ant, Labauchena daguerri continued. This ant is quite effective in decimating and ultimately eliminating colonies of the imported fire ant, but the major objective of present research, how to spread the parasite ant, has not yet been reached. Progress was made in overcoming the resistance of imported fire ants to artificial infestation with Labauchena, promising that an effective method may be found. Two other potential parasites, a mite and a beetle have also been found.

4. Face Flies (Cattle and Horses). At Lincoln, Nebr., Aleochara tristes, an imported parasite-predator of the face fly, and A. bimaculata, a native species, utilize the same habitats in Nebraska pastures and apparently compete for food. A host preference study indicated the order of preference for tristes is face fly, Orthellia caesarion, house fly, stable fly, and horn fly. In the case of bimaculata, the face fly was the least preferred of four dipterous hosts.

Two adults of A. tristes, an imported Staphylinid, were collected from a Nebraska pasture in the spring of 1966. Over 40,000 adult beetles were released in this pasture in the summer of 1965 but adults were not found in October and November. The 1966 collections prove that the species is able to overwinter in nature under Nebraska conditions.

A nematode parasite of the face fly, previously reported from New York, was found in hibernating face flies near Lincoln. In one collection, 34% of the female and 21% of the male flies were infected. Infected females may develop and deposit their first batch of eggs. Rapid multiplication of nematodes in the fly's abdomen prevents further reproduction of the fly. Infested flies, 9 to 16 days old, were able to pass nematode larvae that became infective.

Research was initiated under a grant to Virginia Polytechnic Institute on the effect of native parasites on the natural regulation of face fly populations. Manure that had been artifically infested with laboratory-reared face fly larvae were exposed to native parasites and predators. These preliminary studies showed over the summer an average of 55.6% predation of face fly larvae at Warm Springs, Va., and 45.6% and 48.6%, respectively, at two locations near Blacksburg, Va. This was believed to be largely due to staphylinid beetles. Parasitism of face flies averaged less than 1%, although up to 30% parasitism by Aphaereta pallipes was observed in late

September and early October. Although the face fly is an introduced pest, it appears that native natural enemies have started to attack the face fly.

5. Stable Flies (Cattle and Horses). Tests were conducted in cooperation with the University of California to determine the effects of three species of fungi on stable flies. The fungi were Aspergillus flavus var. columnaris, Beauveria bassiana and Metarrhizium sp. Seven days after treatment, all the Beauveria treated flies, 31% of the control flies, 59% of the Aspergillus treated flies, and 39% of the Metarrhizium treated flies were dead.

D. Insect Sterility and Other New Approaches to Control

1. Mosquitoes. At Gainesville, Fla., research was continued on the evaluation of the sterility principle for control of mosquitoes. Males of Culex pipiens quinquefasciatus exposed as 1-day-old pupae to 7 to 12 kr of gamma irradiation were not completely sterilized. Untreated females mated to these males showed 87 to >99% sterility. Females irradiated with doses of 2 to 6 kr laid fewer eggs than normal when mated with untreated males. As the dose increased the egg production decreased. Doses of 7 to 9 kr prevented oviposition. Exposure to gamma irradiation reduced the mating competitiveness of the males. Aedes aegypti exposed as third instar larvae failed to pupate after exposure to 4 kr. Adult emergence was good following an exposure to 1 kr, but the sterility was low.

A study was conducted to determine the effect of exposure to the chemosterilant apholate in a sugar-water bait on a natural population of Anopheles quadrimaculatus infesting a cow shed. While 78% of the females that fed on the bait were sterile and 67% of them failed to oviposit, there was no clear indication the baits reduced the number of adult mosquitoes in the treated area.

Studies have been conducted to evaluate dust formulations of chemosterilants against mosquitoes. Technical apholate alone gave almost complete sterility of male Culex pipiens quinquefasciatus. Dusts containing 75% apholate on pyrophyllite caused 98% sterility, but mixtures of 50, 25, or 10% apholate on pyrophyllite produced less than 90% sterility. The sterility obtained with undiluted apholate was retained for at least 11 days, and the sterilized males were about equally competitive with untreated males in mating with untreated females. Males of Anopheles quadrimaculatus were also successfully sterilized with undiluted apholate, and these males competed favorably with untreated males for untreated females under laboratory conditions. However, sterile males of both species that were reared in the laboratory and released into a normally reproducing population in a large outside cage did not compete favorably with normal males.

Dust formulations of tepa and metepa have been evaluated against C. pipiens quinquefasciatus under laboratory conditions. Tepa produced 100% sterility at 0.5% in pyrophyllite, the lowest concentration tested, and metepa gave >99% sterility at the same concentration.

The colony of Aedes aegypti which developed resistance to apholate through laboratory selection is more than 20 times as tolerant to apholate as the regular colony. This strain shows little, if any, cross-resistance to tepa but has a 3- to 4-fold cross resistance to metepa. Resistance to apholate is less in the adults than the larvae.

At Corvallis, Oreg., research was also continued to evaluate the sterility approach for control of mosquitoes. Tests with a new cobalt-60 source confirmed dosages of radiation previously found effective in sterilizing Culex tarsalis, but the data suggest there is sometimes more than one mating by some females. Tepa, hempa, and another chemosterilant were tested as possible sterilants for C. tarsalis larvae. Some sterility was obtained but exposure of the larvae resulted in poor survival of the adult mosquitoes.

Research was conducted under cooperative agreement with the University of Florida to study sterilization of a mosquito by irradiation. Irradiation dosage necessary to produce sterility was determined for adult Culex pipiens quinquefasciatus. A sterilizing dose and a lethal dose was determined for the eggs, but dosages tested with larvae showed lethality without sufficient sterility.

Research has continued under PL-480 at the University of Cairo in Egypt on the control of mosquitoes by chemosterilants. The dosage of chemosterilant needed to produce sterility has been established for Anopheles pharoensis.

2. House Flies. Research was continued at Gainesville, Fla., on the development of sterilization techniques for house fly control. One thousand and four chemicals were screened for chemosterilant activity, and 80 of these caused complete sterility in adults. Twenty-one compounds produced 99 to 100% sterility in males when the insects were offered a choice of treated and untreated food.

In an attempt to develop a simple method of sterilizing house flies with chemosterilants, pieces of polystyrene foam have been immersed in various concentrations of metepa or tepa and used to cover house fly pupae to a depth of 2 3/4 to 7 inches. These treatments have produced high sterility in the insects that emerged through them. Applications of 5 and 7.5% metepa produced 97 to 100% sterility while 5% tepa caused 100% sterility.

Male house flies have been successfully sterilized by confining them in cages with females that were carrying pads impregnated with Olin 53330 on their abdomen or that had been treated directly on the dorsum of the abdomen with Olin 53330, metepa, or tepa. The males were almost completely sterile for 15 days. When the chemicals were applied directly to the abdomen, males exposed to virgin females treated with Olin 53330 or tepa became completely sterile for at least 15 days.

Triphenyltin acetate has been found an effective chemosterilant in laboratory tests when offered to mixed sexes of house flies at concentrations of

0.01%-0.025% in a dry sugar bait if the insects had a choice between treated and untreated food. When only treated food was furnished the flies usually were killed before they could lay.

At Corvallis, Oreg., research was continued on methods of sterilizing the little house fly. The median sterilizing dose is about 600 r for females and 400 r for males when irradiated as pupae. When irradiated in the adult stage, 3 to 4 days old, greater doses were required; 900 r for females and 600 r for males.

At Beltsville, Md., investigations of physical methods for fly control were continued in cooperation with the Agricultural Engineering and Animal Husbandry Research Divisions. Mechanical devices, including electrocutor grids, the grids plus lights, and lights plus toxicant (attractant-toxicant devices) all proved ineffective for control of house flies outdoors in cattle pens. Unlighted devices were no less effective than lighted ones, indicating random flights to all units and not attraction. Attractant-toxicant devices of three types tested inside barns killed slightly larger numbers of flies.

Research has continued under PL-480 at the University of Cairo, Cairo, Egypt on the control of house flies by chemosterilants. The dosage of chemosterilant needed to produce sterility has been established for Musca domestica vicina.

3. False Stable Fly (Cattle).

In Oregon, studies on the effects of gamma radiation on false stable fly, Muscina stabulans were initiated. Late stage pupae were exposed to 1750 r and 5250 r. Females given the shorter exposure were incapable of ovipositing. The sterilizing exposure for males is near 5250; less than 1% of the eggs from treated males-normal females crosses hatched.

4. Screw-worm (All livestock except poultry)

Of 359 compounds screened as chemosterilants in Mission, Texas, 42 caused sterility in one or both sexes of screw-worm flies when fed to adults. Evaluation of chemosterilants passing screening tests showed that ENT-50441 sterilized males and females when fed orally or applied topically.

A homologous series of compounds of formula $[CH_2CH_2\ NCONH]_2(CH_2)_n$, when n is 4 to 10, was tested topically and orally against adult screw-worm flies. Lengthening the aliphatic chain tended to decrease toxicity; lengthening the aliphatic chain beyond $n = 7$ tended to decrease chemosterilant action.

Males sterilized by tarsal contact with a residual film of ENT-50838 on glass (20 mg/0.09 m^2) or by intrathoracic inoculation (0.5 and 1%) were less competitive sexually than fertile males. Male screw-worm flies sterilized topically with 5, 10, and 15% ENT-50838 were more competitive sexually

than fertile males by a factor that increased as the concentration increased.

ENT-50838 sterilized males (topical) first mated when 5 days old and others when 12 days old and thrice more at 48-hr intervals were completely sterile at all matings. Age and repeated matings adverseley affected the fertility of control males.

Screw-worm flies were treated topically with graded doses (0.1 to 10%) of ENT-50838. The rate of induced dominant lethal mutations was low at 0.1% increased rapidly to 0.5%, and leveled off at 1% in both sexes.

Screw-worm flies of each sex were treated topically with ENT-50838 and maintained at 26 and 37° C. Elevated temperature alone exerted an adverse effect on egg hatchability. However, elevated temperature appeared to have no further adverse effect on the fertility of flies also treated with chemosterilant.

The competitive mating ability of young and old chemosterilized screw-worm males was compared. Old males (15 days) treated with ENT-50781 or 50838 were less competitive than young males (5 days) similarly treated. Although young males treated with ENT-50781 were at least as competitive as untreated males, old males were slightly less competitive. With ENT-50838, young and old males surpassed untreated males in competitive mating ability. Thus, there was an unfavorable effect owing to age on the mating ability of chemosterilant-treated males. Whereas this factor influenced their competitive mating ability adversely with ENT-50781, it did not with ENT-50838.

Chemosterilant-treated (ENT-50781, 50838) flies contaminated their environment from which other flies acquired chemosterilant and suffered adverse effects of fertility slightly with short exposures (24 hr) and more severely with long exposures (7 days).

Screw-worm flies were treated orally with different concentrations of sugar syrup containing 1% ENT-50781. Egg hatchability increased progressively as the concentration of sugar syrup used as the vehicle for 1% chemosterilant fed to both sexes decreased from saturation to 0.1% of saturation. When the length of time males fed on 1% chemosterilant was increased from 1 to 24 hr, egg hatchability decreased more rapidly if saturated sugar syrup was used instead of 0.1% of saturated sugar syrup.

The effect of ENT-25296 on sperm stored in the spermathecae was determined by treating males topically with a substerilizing dose and mating the males with females that were stimulated to oviposit 3 times at 3-day intervals. Sterility was increased in sperm that was stored by females for 3 and 6 days prior to fertilization of the eggs.

Mating tests with ENT-50990 treated males, when combined at different ratios with fertile males, were less competitive.

The sexual vigor of males treated orally with 0.1% ENT-51254 or 51256 in sugar syrup or topically with 10% ENT-51256 in water was evaluated. Orally administered chemosterilants did not affect male sexual vigor adverseley, but this was not the case with topically applied ENT-51256. Males treated orally with ENT-51254 and 51256 showed that ENT-51254 sterilized males were about twice as competitive sexually, but ENT-51256 sterilized males were about a third less competitive.

5. Horn Flies (Cattle). Studies in Texas indicate that both horn fly pupae and newly emerged adults of the Kerrville laboratory horn fly colony could be sterilized with 3000 r of gamma rays but these flies were not fully competitive sexually. As part of the studies, a preliminary field release of sterile horn flies was made on a semi-isolated herd of cattle at Camp Stanely, Texas. Although cross-fertilization occurred, some viable eggs were obtained during the 6-week study. One phase of the work utilized alternately a non-residual insecticide and sterile-fly release. This combination appeared to be more promising than sterile-fly releases alone.

6. Lice (Goat). Tests in Texas on the effects of Co-60 irradiation on the Angora goat biting louse showed some female lice can withstand a dosage as high as 8600 r but above 4300 r above-normal mortality occurs. Preliminary evidence indicates that 3000-4000 r reduces the viability of eggs laid by irradiated females but does not completely eliminate hatching.

7. Ticks (Cattle and Dogs). In Texas, engorged nymphs of the Gulf Coast tick, Amblyomma maculatum, were exposed to three levels of radiation (Co-60) at three intervals during the molting period. A level of 860 r had no effect on molting; levels of 2150 and 4300 prevented molting of nymphs exposed at 1 week after engorging. A few adults from nymphs exposed to 4300 r engorged. Females from nymphs treated with 2150 and 860 r did not produce viable eggs when mated to untreated males. The radiation effect varied according to the sex of the treated tick as males from nymphs treated with 2150 and 860 r produced some viable eggs when mated to normal females.

At Kerrville, Texas, engorged nymphal Rhipicephalus sanguineus, brown dog ticks, were exposed to gamma radiation of 500, 1000, 2500, and 5000 r at 1, 5, 11, and 15 days postengorgement. Nymphs exposed to 2500 and 5000 r at 1 and 5 days postengorgement did not molt to adults. Adults from irradiated nymphs were mated with normal adults, and effects on engorging, egg laying, and hatch of eggs were recorded. A dosage of 500 r had no effect. At 1000 r there was some reduction in size of females, size of egg masses, and percent hatch. At 2500 r, treated females did not lay eggs, but normal females mated to treated males laid a few eggs that hatched. At 5000 r, treated females did not engorge; a few untreated females mated to treated males engorged and laid some eggs that hatched.

8. Tsetse Flies. Under a PASA agreement with AID, research was continued in Salisbury, Rhodesia in cooperation with the Agricultural Research Council of Central Africa on the feasibility of the sterile male technique for the

control of tsetse flies. Seasonal influences were detected in chemosterilization trials. Overall G. morsitans survival was reduced by wind tunnel and contact treatments with tepa and metepa in both the winter and summer seasons; however the major factor influencing survival was season. Competitiveness, insemination, and sterility were not affected by season. Chemosterilization of 1-day old G. morsitans males with tepa in the wind tunnel and by contact exposure resulted in complete sterility and competitiveness. Competitiveness was less than expected in similar trials with metepa, although complete sterility was obtained.

Wind tunnel application of 0.25 ml of 5% tepa failed to sterilize G. pallidipes completely, but 0.5 ml was effective and provided permanent sterility. Exposure of G. pallidipes males to gamma-irradiation resulted in increasing sterility and decreasing survival as the dosage was increased from 4,000 to 16,000 r and as pupal age at the time of treatment was decreased, but complete sterility was rarely achieved. G. pallidipes females, with one exception, were completely sterilized with 4,000 to 8,000 r but survivals were not affected by the treatment. Irradiation of adult males with 8,000 to 16,000 r was not as effective as pupal treatment and resulted in decreased survival.

Irradiation of G. morsitans males when 1 or 6 days old with 8,000 to 15,000 r of gamma radiation from Co-60 resulted in 93 to 97% sterility. Females were completely sterilized with 2,000 to 4,000 r. Both sexes appeared to survive better than the controls. Fractionated dosages of 2,000 X 4, 4,000 X 2, 4,000 X 3 and 5,000 X 3 given one day apart to pupate did not reduce male fertility quite so much as continuous exposure to 8,000, 12,000 and 15,000 r, respectively. Male longevity was better than with continuous exposures, but below the control longevity, and was similar to that expected from a single exposure to 4,000 r.

An interesting finding was that sterile G. morsitans males can act as vectors of trypanosomiasis when the sterilization treatment precedes the infection in the fly; the data suggest that transmission is reduced when the treatment occurs after the fly is infected.

Attractant investigations in the laboratory have not indicated that G. morsitans females produce a chemical sex attractant, but preliminary bioassays resulted in positive male response to lipid fractions from virgin, mature females. Males appeared to respond and attempt mating only after visual attraction to active females, but generally lost interest rapidly if they failed to copulate almost immediately. Painting the eyes of the male reduced mating, but not feeding, in small cages. Removing the antennae of either sex, or the halteres and wings of females, did not affect mating under laboratory conditions.

None of the three large cages at the Chirundu station have provided adequate conditions for prolonged G. morsitans survival. Oxen, warthogs and bushpigs

were used as host animals. Extensive modifications to alter the light patterns within one cage have resulted in improved feeding by the flies, but not survival. In an intermediate size cage, which consists primarily of large cement pipes, survival has been extended to 22 days but 50% of the flies died in the first 5 days. Poor survival also occurred in the vertical cage which encloses two trees. Fly behavior and movement was observed in this cage with the aid of radioactive Tantalum.

Small cages containing G. morsitans were placed inside the large field cages during the trials. Survival was much better within the small cages than in the large volume of the big cages. Small cages of flies in the controlled environment room and in the varying climate of the wicker cage have demonstrated good survival and reproduction rates, although flies released in the large volume of these two cages do not survive well.

Attempts to concentrate a breeding focus of Glossina have not been successful. After two separate one-year trials, involving the positioning of cattle in favored tsetse areas, no permanent fly buildup has resulted.

Population surveys showed that G. morsitans density on Long Island, the site selected for a small field release experiment, remained relatively stable from November to May, increased rapidly through August-September, and then to fall sharply to return to the November levels. Population densities were estimated at 3700 to 5300 per square mile depending on the season. Separate field trials demonstrated that marked G. morsitans males dispersed sufficiently to satisfy the assumptions of the statistical model used to esimate the population on Long Island.

9. Mites (Poultry). Research on the evaluation of chemosterilants as a means of control for the northern fowl mite was initiated under grant support at the University of Mississippi. The internal morphology of the reproductive system was studied. Isolation apparatus to confine the test birds and mites were constructed so that at least two compounds can be tested simultaneously. Three candidate chemosterilants are now being evaluated.

E. Insecticide Residue Determinations

1. Residue Studies. In Texas, studies were conducted to determine whether certain insecticides applied to dairy cows would contaminate the milk. Two dairy cows were treated with a 2% or a 5% malathion solution in xylene. The insecticide was applied with a single nozzle, low-volume, automatic sprayer adjusted to deliver 1 ml of solution in a mist across the backs of the animals, covering an area about 30 cm wide extending from the withers to the loin. The animals were treated twice daily after milking, for a period of 21 days. Samples of milk were taken before treatment on the first day of the test and at intervals up until 21 days after the treatment started. Analysis by a method sensitive to 0.001 ppm of malathion revealed no residue of this insecticide in the milk.

In Texas, two dairy cows were sprayed twice daily for 28 days with 1 ml of solution of ronnel in xylene. A 5% soultion was applied to one cow and a 10% solution to the other. Residues of ronnel were detected in the milk from both cows one day after spraying began and increased to a maxima of 0.14 and 0.35 ppm, respectively.

In Texas, two dairy cows were exposed to a back rubber treated with the recommended amount of 2% ronnel in oil (1/2 gal/10 ft) twice daily for 14 days. The back rubber was then retreated and used for another 14 days. Milk samples were taken after 1, 3, 5, 7, 10, and 14 days exposure. Two other cows were exposed to a back rubber treated with twice the recommended amount of 2% ronnel solution in oil twice daily for 14 days. Milk samples were taken at the same intervals as before. Ronnel was not detected in any of the 20 milk samples taken from the cows using the back rubbers treated with the recommended amount of ronnel. In the case of the cows exposed to the rubbers treated with twice the recommended amount of ronnel, their milk contained 0.002 and 0.007 ppm of ronnel after 1 day's exposure and 0.003 and 0.002 ppm after 3 days. No residues were detected during the rest of the period of study.

In Maryland, no residues of dimethoate or its oxygen analog were found in the milk of cows given oral administrations daily for 14 days of 0.50 mg/kg of dimethoate and 0.05 mg/kg of its oxygen analog in capsules. When the doses were doubled, oxygen analog ranging from 0.001 to 0.125 ppm was found in the milk but no dimethoate was detected. Milk samples were taken throughout the entire time.

In Georgia a gas chromotographic procedure utilizing a flame photometric detector was developed for the determination of residues of coumaphos and its oxygen analog in milk and feces. This method was used in a study of the residues resulting when coumaphos (0 to 48 ppm in air-dried feed) was fed to dairy cows to study its activity against house fly larvae emerging in the cows' feces. Neither coumaphos nor its oxygen analog was found in the milk of the cows. Coumaphos appeared in the feces.

A gas chromatographic procedure was also developed in Georgia for determination of residues of Shell SD-8447 (2-chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate) in milk and feces from dairy cows that had been fed the insecticide in a study of its activity against house fly larvae emerging in the cows' feces. Milk from cows fed amounts of SD-8447 ranging from 0 to 844 mmg contained no detectable residues of SD-8447 or its hydrolysis product. In the feces, from <0.013 to 0.498 ppm (dry basis) of SD-8447 was found, but its hydrolysis product was not detected.

Further residue studies in Texas with Shell SD-8447 were conducted. Emulsion sprays of Shell SD-8447 at 0.125, 0.25, and 0.5% concentration were applied to cattle one time by normal spraying procedures. Analyses were made of omental, renal, and subcutaneous fat, and also of muscle, heart, liver,

kidney, spleen, and brain when residues of 0.002 ppm or more of SD-8447 were found in the fat. The maximum residue found was 0.108 ppm in omental fat of a calf that received an 0.5% spray and was slaughtered 1 week after spraying. Two groups of cattle were then sprayed to saturation with an emulsion containing 0.25% SD-8447. All tissues were analyzed from one group at 1 and 3 weeks after treatment. For the other group only the fat samples were analyzed at 1, 2, and 3 weeks after treatment. The only residues found were in the fat and these were no longer detectable (<0.002 ppm) by 3 weeks after treatment.

Analyses were made for residues of the oxygen analog of Dursban in the body tissues of cattle after three dippings at 2-week intervals in 0.05% Dursban emulsion. Seven days after the treatment 0.02 ppm of the oxygen analog was found in the omental fat and 0.03 in the renal fat. No measurable residues were found in the muscle, spleen, or brain and none were detected in the omental fat and renal fat 10 weeks after treatment.

In Texas a method was worked out for the determination of Stauffer R-3828 (S-(p-chloro-α-phenylbenzyl) 0,0-diethyl phosphorodithioate and its oxygen analog in the body tissues of cattle. Results obtained thus far show that a calf which received 5 mg/kg of R-3828 daily for 14 days had 40.4 ppm of R-3828 and 0.73 ppm of the oxygen analog in the omental fat, while another calf receiving 10 mg/kg daily had 133.0 ppm of R-3828 and 2.50 ppm of the oxygen analog. In a metabolic study of the compound, a single dose of 100 mg/kg of C^{14}-labeled R-3828 was given by capsule to a calf. Fat samples taken from this calf contained 118.0 ppm of R-3828 and 0.27 ppm of its oxygen analog 13 days after treatment and 2.0 ppm of R-3828 and less than 0.005 ppm of the oxygen analog 63 days after treatment. The compound produced the highest residues in the fat of cattle of any organic phosphorus insecticide thus far studied. An 8 week feeding test with this insecticide was completed.

Animals receiving 5 mg/kg of R-3828 daily had a residue of 81 ppm in the fat at the end of the 8 week feeding period; this residue decreased to 10.4 ppm 42 days after feeding ceased. Animals receiving 10 mg/kg daily had 170 ppm in the fat at the end of the feeding and 16.5 ppm 42 days after feeding ceased. The fat of the animals receiving the lower dosage contained 2.10 ppm of the oxygen analog of R-3828 at the end of the feeding period. The fat of those receiving the higher dosage contained 2.0 ppm.

In Maryland, cuts of pork and pork products from hogs fed diets containing heptachlor (2.8 ppm) and DDT (34 ppm) were analyzed for pesticide residues. In the cooked meats heptachlor epoxide residues were found ranging from 0.6 to 0.8 ppm in lean meat, 0.8 to 3.4 ppm in fat and drippings, 0.1 to 0.2 ppm in ham, and 1.3 to 2.4 ppm in bacon and bacon drippings. The combined residues of DDT and its analogs ranged from 2.2 to 12.8 ppm in lean meat, 4.8 to 19.7 ppm in fat, 1.5 to 3.0 ppm in hams, and 5.6 to 37.2 ppm in bacon and drippings.

In Maryland, beef calves were fed a mixture of dimethoate (0.30 mg/kg body weight) and its oxygen analog (0.03 mg/kg) daily for 14 days. Analysis showed no residues of dimethoate or its oxygen analog in selected organs, blood, or muscles from the calves.

In Washington, residue studies in cattle from the Burns Tassock Moth Project were continued. Adipose tissue of cattle from areas treated with DDT (0.84 kg/hectare) applied in the spring of 1965 was analyzed for DDT and its metabolites. Samples were taken in the fall of 1965 and the spring and fall of 1966. Based on 100% extractable lipids, the ranges and averages of combined DDT and its metabolites were: for the fall of 1965, 6.3 to 16 ppm (average 12 ppm, 9 cattle); spring of 1966, 0.5 to 14.6 ppm (average 5.5 ppm, 10 cattle); the fallof 1966, 0.1 to 4.5 ppm (average 1.1 ppm, 10 cattle).

In Washington, DDT residue studies in wild game were made. In 1964, 500,000 acres of the Salmon National Forest were sprayed with DDT for control of the spruce budworm. Samples of adipose tissue were collected during the 1965 hunting season from 27 deer and 17 elk and were analyzed for combined DDT isomers and metabolites. Based on 100% lipid content, the residue found in the deer ranged from 0.035 to 5.11 ppm (average 1.92 ppm) and in the elk from 0.27 to 3.78 ppm (average 3.07 ppm). This project was in cooperation with the U. S. Forest Service and the Idaho State Fish and Game Department.

In the spring of 1965 DDT was applied at the rate of 0.84 kg per hectare in forests in Oregon and for the control of the tussock moth. Samples of adipose tissue were collected from 17 deer and 1 elk (killed between July 27 and August 31, 1965) and were analyzed for DDT and its metabolites. On the basis of 100% extractable lipids, the residues found in the deer ranged from 0.07 to 31 ppm (average 4.60 ppm). Two control deer killed outside the treated area had residues of 0.15 and 0.13 ppm (average 0.14). The elk adipose tissue contained 1.83 ppm. This project also was in cooperation with the U. S. Forest Service.

In Washington, the persistence of the insect chemosterilant, tepa, in bird mash feed was determined. In 2 samples of the mash the initial levels of tepa were 9.2 and 16.8 ppm, whereas theoretically they should have been 25 and 50 ppm. (These samples were fortified with tepa at Davis, California, and shipped frozen to Yakima. Control samples fortified at Yakima contained the theoretical amount.) Similarly treated samples that had been held in bird cages for 7 days were found to have 0.00 and 0.24 ppm of tepa. This work was in cooperation with the U. S. Fish and Wildlife Service.

F. Attractants

1. Mosquitoes. At Gainesville, Fla., the olfactometer system was adapted for screening of chemicals as mosquito attractants. One hundred and forty-five chemicals were screened. Of these 9 showed some promise as possible attractants. Four of the 9 were propanediols. The procedure was revised to include a second technique with each compound. A flow of carbon dioxide (5-10 ml/min) was introduced with the candidate chemical to determine

whether the addition of the gas would increase the attractancy. Nineteen of 64 chemicals showed some attraction. Five percent or more female A. aegypti were attracted by 4 of the compounds with or without carbon dioxide, by 3 without carbon dioxide and by 12 with carbon dioxide.

Studies at Corvallis, Oreg., were also continued on the attractive principle in log pond waters. Chloroform extracts of both log pond water and cold-trapped odors from these waters were attractive to ovipositing Culex pipiens pipiens. Even the presence of log pond odors caused more oviposition on distilled water than occurred in the absence of the odors, but more egg rafts were deposited by C. p. quinquefasciatus females when they were able to contact log pond waters. In initial tests, chromatographed fractions of log pond water extracts included fractions highly attractive to ovipositing pipiens, quinquefasciatus, and C. tarsalis, and one fraction repellent for the three species. However, in further studies with waters from six different log ponds, the repellent fraction was not found with chromatography. There has been some evidence that the presence of cedar was detrimental to mosquito breeding, but no clear-cut evidence of toxicity was found with extracts of cedar roots, heartwood, or sapwood, and waters holding cedar logs received 12 times as many egg rafts of C. p. pipiens as did the distilled water controls. Water from ponds holding Douglas fir logs received 9 times as many egg rafts as the controls. Chloroform extracts of Douglas fir phloem showed little promise, but extracts of bark produced fractions with attractiveness near that of log pond water extracts. The chromatographed R_f values of extracts of fir bark and log pond waters were comparable, therefore further isolation of the fir bark extracts was made. The acid derivatives were more attractive than basic derivatives, but the attractancy was apparently related to a combination of materials and not necessarily to a single extractable.

At Corvallis, Oreg., research continued with what are believed to be sex pheromones of mosquitoes. Virgin female Culex pipiens quinquefasciatus, C. p. pipiens, and C. tarsalis respond to pheromones of the males of their respective species or subspecies, and also to those of the males or opposite subspecies or the other species. To some degree, males are also attracted. Female quinquefasciatus and pipiens given a 6-day opportunity to mate also showed attraction to males of the three species in the olfactometer.

2. House flies. Research was continued at Corvallis, Oreg., on the sex pheromone reported earlier in the house fly. Response of flies was found to be somewhat variable, but the response occurred with wild flies, as well as laboratory strains, and colonization of a wild strain for 12 generations produced no loss or gain of the pheromone. In other tests with individual flies it was noted that apparently the threshhold of pheromone needed to stimulate mating varied with individual flies. A strain of blind flies still responded to the pheromone, although with normal flies a visual stimulus (such as a dead fly or object the size and rough shape of a fly) usually enhances the response. Efforts to purify, characterize, and identify

the pheromone are continuing as a joint project with chemists at Beltsville, Md. Over 250 independent materials, purified fractions, and extracts have been tested, but the pheromone has not yet been isolated. The pheromone is apparently a recognition mechanism, that identifies the opposite sex of its own species for the male house flies, which will initiate strike against any fly their size, including other males, but remain to copulate only with female house flies which, of course, have the pheromone.

3. Horn flies. Research was initiated under grant support at the New Mexico State University, Las Cruces, on the response of the horn fly to extracts of animal tissues and to putrefaction products. A laboratory colony was established, two types of olfactometers were built and are being evaluated. Preliminary tests indicate that water and petroleum ether extracts of bovine hide and hair or water extracts of skatole and indole were not attractive to newly emerged flies.

4. Screw-worm (All livestock except poultry). In Texas 231 chemicals were screened as screw-worm attractants. None were found to be as effective as the standard liver bait.

Differences have been found in the response of unmated females of 2 laboratory strains of screw-worm flies (from Florida and Mexico respectively) to a male pheromone from each of these strains. The Florida strain females, under colonization for a period of 10 years, responded to the male pheromone of both strains with characteristic aggressive behavior. Females of the Mexican strain (colonized about 2 1/2 years) did not respond to the pheromone from either strain. It is postulated that the difference in response by females of both strains may be explained, at least in part, by selection brought on by some of the techniques used in mass production over extended periods of colonization.

Limited tests with odors collected from screw-worm infested sheep and extracted with ether have indicated that the method of presentation affects the overall response by male and female screw-worm flies. In laboratory tests fly catches on the wheel-type olfactometer were found to be predominately male (86%), while flies captured in wound odor baited traps attached to a sheep were found to be only 4% male. These tests were run simultaneously. Fly catches in field tests made with irradiated flies and the wound odor-trap attached to sheep have been 100% female. The fact that controls (sheep + odorless trap) captured a few female screw-worm flies (range 0 to 5) is interesting and suggests that an "animal factor" is involved in female screw-worm response.

G. Insect Vectors of Animal Diseases

1. Anaplasmosis (Cattle). In 1966 studies in Mississippi were designed to evaluate the relative importance of Culicidae and Tabanidae as vectors of bovine anaplasmosis. Three splenectomized anaplasmosis negative steers and one anaplasmosis carrier steer were exposed continuously outdoors to attack

by horse flies and mosquitoes for a six-week period. A similar group was exposed in a screened building which excluded the horse flies. Two of the three susceptible animals in the outdoor exposure developed anaplasmosis. None of the animals in the screened group developed anaplasmosis. Groups of susceptible animals exposed for two-week periods during the six-week study indicated that transmission of the disease occurred primarily during the second two weeks of the test.

Collections from bait animals, light traps, and animal-baited insect traps showed that the main mosquito species involved were Psorophora confinnis and Anopheles quadrimaculatus; the main tabanid species were Tabanus subsimilis, T. lineola, and T. fuscicostatus. The periods of peak populations of the Culicidae occurred during the last two weeks of the test period, and of the Tabanidae during the first two weeks of the test period.

The data accumulated from the 1965 and 1966 studies indicate that mosquitoes probably do not and that horse flies do play a major role in the transmission of anaplasmosis.

Observations made on bait animals during the study in 1966 indicate Hippelates or eye gnats need to be investigated for their role in anaplasmosis transmission. These insects are associated with horse flies at the time horse flies are feeding on the animal. The Hippelates are apparently attracted to the blood from the wounds made by the horse flies and feed readily on this blood. Hippelates were present on the animals in the pasture but were not on the animals within the screened building. The main peak of activity for these flies occurred during the period when transmission was believed to have occurred in the field.

Cooperative studies with the Animal Disease and Parasite Research Division continued at Beltsville, Maryland, on the transmission of bovine anaplasmosis, but at a much reduced level. Attempts are still being made to establish naturally infected colonies of ticks from anaplasmosis enzootic areas. One transmission trial was conducted with the tropical horse tick, Dermacentor nitens. The ticks were allowed to feed on a calf which was in the acute stages of Anaplasma marginale infection and the F_1 progeny of these ticks were tested on a splenoctimized calf. Anaplasmosis was not transmitted. Further transmission trials with this species are planned.

2. Equine piroplasmosis. Cooperative studies with the Animal Disease and Parasite Research Division were continued at Beltsville. A colony of tropical horse ticks, Dermacentor nitens, infected with Babesia caballi has been established. This colony transmitted the disease to susceptible horses when tested at each of 8 successive generations. Babesia caballi appears to be transmitted by D. nitens early in the nymphal feeding period. Test horses developed patent parasitemias 3 to 6 days after the larval-nymphal molt. The parasitemias persisted at varying levels in the test horses throughout the period when the adult ticks were feeding thus insuring development of B. caballi in the replete females and infection of the following generation of ticks.

Studies of the life cycle of B. caballi in D. nitens indicate that development may begin 48 to 72 hours after the parasites are ingested. Babesia caballi invades the gut epithelial cells of the tick and undergoes multiple fission releasing motile vermicular shaped parasites which invade developing ova and other tissues of the tick. Parasite development appears to be quite slow in the eggs, however late in the incubation period, or early in the larval feeding period, the multiple fission cycle is repeated releasing vermicular forms, some of which invade the salivary glands of the larvae or nymphs. Multiple fission occurrs again in the salivary glands resulting in large numbers of small (1.5 to 3.0 μ) oval or piriform parasites which are infective to horses. Additional studies on the precise mode of development, predilection of tissue, and longevity of B. caballi in D. nitens are being continued.

3. Bluetongue (Sheep, Cattle, Goats, and Ruminant Wildlife). Studies have continued on the biology of insect vectors of bluetongue disease of sheep and cattle and the role of insects in the transmission of the disease in cooperation with the Animal Disease and Parasite Research Division at Denver, Colo. It was reported previously that flies (Culicoides variipennis) fed on vaccinated sheep (egg-attenuated modified live vaccine - Blucine) were able to pick up the virus. The virus multiplied in the insect and a virulent form of the disease was passed to susceptible sheep by the bite of these flies. This research indicates that vaccination with modified live virus vaccines, at least for bluetongue, should be done only in areas where the disease is endemic, since its use in other areas may only serve to spread the disease among livestock or setup a foci of infection in wildlife. It was also found that the bite of a single infected fly was sufficient to transmit the disease from sheep to sheep. About 6% of flies fed on vaccinated animals became infected compared to 10% of those fed on animals inoculated with virulent virus. In these tests, the serial transmission of the vaccine virus by fly bite was shown through a series of several sheep.

The occurence of several strains of bluetongue virus in the United States has been shown by the Animal Disease and Parasite Research Division. Sentinel sheep established in the field at an epizootic of bluetongue in cattle in Montana apparently picked up some immunity to one strain of virus (BT OX 254). This immunity did not protect 2 of the 3 animals against challenge with the standard BT 8 virus. Research is continuing on the cytological and physiological changes that occur in the structure of the salivary gland of C. variipennis almost immediately after flies are given a blood meal.

The methods used to maintain the colony of C. variipennis have been drastically changed. Primarily the larval medium now consists of a bacterial broth nourished with commercially available products, rather than relying on cow manure as the primary ingredient.

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AREA NO. 14. INSECTS AFFECTING MAN, HOUSEHOLDS, AND INDUSTRIAL ESTABLISHMENTS

Problem. Insects, ticks, and mites are responsible for widespread human misery and certain insects cause heavy losses of food and materials in households and industrial establishments. Many of the same or closely related insects which affect man are also important pests of livestock, thus research on insects in relation to man and to livestock is mutually advantageous. Certain arthropods are vectors of major diseases which annually cause the deaths of millions of humans. Mosquitoes, for example, transmit malaria, dengue, encephalitis, yellow fever, and filariasis. Some of these diseases, as well as other arthropod-borne diseases, occur and are potentially serious problems in the United States but most of them are of more concern in other parts of the world where troops and civilian personnel of the United States are maintained. The military agencies have for many years depended on the research competence in agriculture for answers to their military insect and insect-borne disease problems. Attacks by insects, ticks, and mites frequently interfere with farm and forest work, reduce or destroy the value of recreation areas, and even make certain areas uninhabitable. Property values are often depressed and development prevented by hordes of annoying pests. Mosquitoes, bed bugs, and fleas are frequently serious annoyances in homes. Other household insects are of economic importance in homes and industrial establishments because they damage foods, fabrics, and other materials, causing losses of millions of dollars annually. There is a great need for safe, economical insecticides and satisfactory methods for their application that could be used quickly and effectively to control local infestations or outbreaks of pests that annoy man in the field or at home, especially where there are threats of disease epidemics. Improved means for controlling mosquitoes, sand flies, gnats, the imported fire ant, and similar pests should receive particular attention. More efficient repellents are needed to protect humans, particularly when other means of control cannot be employed. Special efforts should be made to develop systemic materials which when taken orally would repel or prevent insects from biting. Sanitation, habitat management, and other noninsecticidal methods of control should be reappraised, and biological control, especially with insect pathogens, needs to be fully explored. New approaches to control including chemosterilants, antimetabolites, attractants, and radiation require intensive investigation. Studies should be undertaken on the biology, ecology, physiology, and genetics of many important pests affecting man and the household in order to find weak points in their life cycles which might be utilized to improve control efficiency.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing program involving basic and applied research on the biology and control of insects and related arthropods affecting man,

households, and industrial establishments including studies on mosquitoes, gnats, house flies, human lice, bed bugs, fleas, ticks, mites, cockroaches, ants, and other pests. The Federal scientific effort devoted to research in this area totals 26.0 scientific man-years of which 5.5 is concerned with basic biology, physiology, and nutrition; 6.5 with conventional insecticidal control methods; 3.0 with insect predators, parasites, and pathogens; 5.5 with insect sterility and other new approaches to control; 4.0 with attractants and repellents; and 1.5 with program leadership.

The major portion of the program is conducted at Gainesville, Fla.; the remainder at Corvallis, Oreg.; Fresno, Calif.; Lake Charles, La.; and Beltsville, Md. Close cooperation and evaluation of research needs and data are maintained with the Department of Defense through the Armed Forces Pest Control Board concerning studies on insects important to military personnel. Research funds supporting 3 scientific man-years have been transferred from the Department of Defense to support research in this area. Cooperation is maintained with the World Health Organization on studies for developing new insecticides and other methods of control of insects affecting man. The World Health Organization provides financial support(0.5 scientific man-years) for studies at Gainesville, Fla., on the development of residual insecticides for mosquito control.

Federal support in research grants, contracts, and extended cooperative agreements totals 2.2 scientific man-years, of which 0.5 is devoted to basic biology, physiology, and nutrition; 0.8 to conventional insecticide control methods; 0.2 to insect parasites, predators, and pathogens; 0.5 to insect sterility;and 0.2 to attractants and repellents. The extramural research is conducted at the following institutions: University of California at Davis; University of Georgia, Athens; University of Southwestern Louisiana, Lafayette; McNeese State College, Lake Charles; New Mexico State University, Las Cruces; and Virginia Polytechnic Institute, Blacksburg.

Additional research is carried out under P. L. 480 grants including S9-ENT-7, "Investigations of natural enemies of ants", Ministeria de Ganaderia y Agricultural, Montevideo, Uruguay; F4-ENT-6, "Studies for the control of house flies and mosquitoes by means of chemosterilants in Egypt", Department of Entomology, University of Cairo, Egypt; and A10-ENT-11,"Action of repellents on mosquitoes, fleas, ticks, and mites", Department of Entomology, Israel Institute for Biological Research, Ness-Ziona, Israel.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 31.2 professional man-years is devoted to this area of research.

PROGRESS - USDA AND COOPERATIVE PROGRAM

A. Basic Biology, Physiology, and Nutrition

1. Mosquitoes, sand flies, and gnats. At Gainesville, Fla., studies

are being conducted to obtain biological information needed to accurately evaluate the potentialities of chemosterilants against mosquitoes. Data obtained recently indicate females of Culex pipiens quinquefasciatus may mate more than once if combined with large numbers of males. However, sperm from secondary matings are utilized only if the matings occur within a few hours of each other. Normal males have been found capable of mating 1 to 2 females per night and males sterilized with apholate can mate about 1 per night.

Additional information has been collected through the action of P^{32} in mosquitoes. Tests have shown that Anopheles quadrimaculatus larvae pick up more radioactivity when exposed to P^{32} in distilled water than in infusion water. Increasing the dosage and exposure time increased the activity in the insects but also increased the mortality that occurred. There is a direct relationship between P^{32} activity in the male and the amount found in females inseminated by these males.

At Lake Charles, La., studies were continued on the biology of salt marsh and rice field mosquitoes. Tests were continued with the screened and unscreened enclosures, in studies now continued for more than 2 years. Initially, it appeared that most of the eggs had hatched by the end of the first year. By then, the hatch of eggs was smaller in the screened enclosures than in the unscreened ones. However, during the second year some hatching of Aedes sollicitans, A. taeniorhynchus (2 salt-marsh mosquitoes) and Psorophora confinnis (major rice-field species) continued to occur, even on the 28th flooding. A comparison of eggs laid in the spring and fall suggests that warm weather species A. taeniorhynchus and P. confinnis, especially the latter, lay some egg batches in the late fall. Most of the eggs are in a deep diapause state and are less hatchable than egg batches laid in the spring. Apparently A. sollicitans eggs do not diapause in the Gulf Coast region. No hatching differences were noted between spring and fall eggs with this species. Ninety-three water samples analyzed from habitats of salt-marsh Aedes spp. and P. confinnis showed that salt concentrations that produced confinnis averaged one-third to one-half those that bred A. sollicitans and A. taeniorhynchus. No pH correlation was noted. In 24 oviposition preference tests, P. confinnis showed a preference for low salinity compared with A. sollicitans and A. taeniorhynchus, but all three species showed definite preferences for increasing concentrations of chloride salts. In further tests, the salt-marsh species sollicitans and taeniorhynchus showed no preferences among chloride salts, but sollicitans avoided sodium carbonate and sodium bicarbonate and these salts were detrimental to sollicitans eggs. Sollicitans eggs hatched in water with a sodium chloride concentration as high as 6.6%. A. taeniorhynchus eggs hatched in water containing as much as 2.0% chloride and P. confinnis eggs hatched at 1.0% chloride.

At Corvallis, Oreg., research continued on the biology of mosquitoes. Aedes dorsalis and A. melanimon are difficult to separate taxonomically. The same situation occurs with Aedes excrucians and A. aloponotum. It is

possible that differences within these complexes may be physiological and due to the habitat. An attempt is being made to establish a colony of A. dorsalis and field sites have been chosen for ecological studies of larval requirements or preferences of dorsalis and melanimon. A seasonal decline in concentration of alkali metals in the water samples from these sites was noted, but further studies will be needed before any conclusions can be drawn. In further biology observations, it was noted that larvae of Mansonia perturbans either develop unevenly or the egg-laying season extends over a considerable period. Aedes increpitus is a fall species. The earliest recorded brood occurred in October, before any killing frost. Thus, exposure to freezing is apparently not a hatching requirement. The commonest species of mosquito breeding this year in log ponds was Culex peus, with lesser breeding of C. p. pipiens, C. tarsalis, and Culiseta incidens in most localities. However, Culiseta incidens was the dominant species at one rural locality and the proportion of pipiens was higher in the urbanized community of Eugene, Oreg., than in more rural settings. A factor in undefatted liver is toxic to larvae of Culex tarsalis. Extracting fat solubles yields a product suitable for larval food.

Captures of insects feeding on a horse showed black flies biting all day long, but highest catches were in the late afternoon. Culicoides gnats were most numerous in the morning. Anopheles freeborni, Culiseta inornata, and Aedes dorsalis fed during the day. Studies were initiated to determine which Oregon species of Culicoides gnats feed on poultry.

Research has continued under two contracts at the University of Southwestern Louisiana and McNeese State College. Mosquito breeding was light around Lafayette, La., particularly in the last half of 1966, compared to previous years. The same situation occured around Lake Charles, La. Several heavy broods of larval mosquitoes failed to produce comparable populations of adult mosquitoes, suggesting some biological control agent was at work attacking the larvae.

Research was initiated under a grant to Virginia Polytechnic Institute on the biology of Culicoides gnats. Initial studies indicate that man is more attractive than small mammals or birds and that the standard New Jersey light trap (used for mosquitoes) and an animal-baited trap were the best of several designs tested for trapping these gnats.

Research was initiated under a grant at Louisiana State University, Baton Rouge on the determination of host animals of mosquitoes in certain areas of Louisiana. Blood was collected from species of birds, mammals, amphibian and reptiles. Antisera were prepared and tested for specificity for all animals collected. Preliminary results indicated that of 8 mosquito species collected per 4 parishes, the majority had obtained blood meals from beef, a few from horses or swine and an occasional one from rabbits and deer, while a few have fed on two hosts.

2. Horse flies - Also in research under a grant at Louisiana State University, on the determination of host animals of tabanids in certain areas of Louisiana, blood was collected from species of birds, mammals, amphibian and reptiles. Antisera were prepared and tested for specificity for all animals collected. Preliminary results indicated that of the 7 species of tabanids collected from 6 parishes the majority had fed on beef, a few on horses and one each on a rabbit and a deer.

3. Yellow jackets - Research to determine the flight range of yellow jackets was conducted at Corvallis, Oreg. On 3 successive days, 1 liter of honey-water mixture labelled with P^{32} at the rate of 1 uc/ml was placed in a protected container near a nest of Vespula pennsylvanica. The yellow jackets consumed the entire liter within 24 hours on all 3 occasions. Nine hundred and ninety labelled yellow jackets were recovered at points up to 3000 feet (0.58 mile) from the nest. Recovery was by traps baited with 2,4-hexadienyl butyrate, the standard lure for yellow jackets. The first labelled specimen was caught within 24 hours, and the last one 24 days later. Thus, either the yellow jackets are able to detect and respond to the lure for at least a half mile, or the normal forage flight of a yellow jacket is as much as half a mile.

B. Insecticidal and Sanitation Control

1. Mosquitoes - At Gainesville, Fla., the search for new and safer insecticides for mosquito control was continued. The most effective materials screened against larvae of Anopheles quadrimaculatus were experimental compounds ENT 27386 and ENT-27444 which gave 100% control at 0.01 ppm. Five other commercial compounds killed all of the larvae at 0.025 ppm.

Comparative tests conducted with the standard larvicide technique have shown Dursban and Abate to be two of the most efficient larvicides that have ever been developed for the control of Aedes taeniorhynchus. They have been 2 to 3 times better than parathion, the most effective compound tested previously and considerably more effective than fenthion, malathion, naled, or DDT. In wind-tunnel tests against adult mosquitoes, six insecticides were evaluated that appear to be as effective as the malathion standard. Eleven synergists have also been found that increased the toxicity of Bay 39007 at least 2 times.

The development of ultra-low volume sprays has received major attention at the Gainesville, Fla., laboratory. Of the materials tested so far naled, fenthion, and Bay 39007 have proven to be more effective than malathion. A comparison of ultra-low volume and conventional sprays showed naled to be about equally effective in both types of application. Fenthion, however, was about 20% less effective in an ultra-low volume spray than in a conventional spray 6 hours after application, but both methods of application caused about the same reduction after 24 and 48 hours. Malathion was

slightly less effective in an ultra-low-volume spray than in a conventional spray regardless of the time interval after treatment.

Bay 39007 has been found more effective than malathion in an aerial fog against natural infestations of adult salt-marsh mosquitoes, Aedes taeniorhynchus and A. sollicitans in Florida.

Additional research has been conducted with a nonthermal aerosol generator developed to disperse insecticides from the ground. Fenthion, naled, Bay 39007, Bay 41831, Schering 34615, Dursban, and Shell SD-8211 and an experimental compound, ENT-27334, have been highly toxic to adult females of Aedes taeniorhynchus, Anopheles quadrimaculatus, and Culex pipiens quinquefasciatus in tests with this equipment. When thermal and nonthermal aerosol applications of DDT and malathion were compared, the two pieces of equipment produced similar mortalities. DDT at 16% was ineffective against Aedes taeniorhynchus and Culex pipiens quinquefasciatus but produced 89 to 100% mortalities of Anopheles quadrimaculatus. Malathion at 4% produced 64 to 99% kill for 300 feet against all 3 species.

The cooperative testing program of military entomologists and personnel of the Gainesville, Fla., laboratory was continued in Thailand. Fogs containing 1, 2, and 4% malathion that were used against natural populations of Culex p. quinquefasciatus caused reductions of 67 to 74%. Mortality as high as 95 to 99% occurred amoung mosquitoes collected from the treated area and held in cages for 24 hours after the treatments were applied. Abate was found highly effective against larvae of Aedes aegypti in water being stored in concrete water jugs. In tests where the water level remained static, Abate in an emulsion formulation killed all larvae added to the treated water for 34 weeks at a concentration of 1 ppm, 19 weeks at 0.1 ppm, and 20 weeks at 0.05 ppm. Abate in granular formulations killed all larvae for 34 weeks at 1 ppm and for 22 weeks at 0.1 ppm. When 15 gallons of treated water were removed and replaced with untreated water each week, the toxicity of the treatments was reduced only slightly. Concrete pellets impregnated with Abate were also effective.

Larval selection with DDT for nine generations increased the resistance in a strain of Anopheles quadrimaculatus, that was already known to possess some resistance, to a level that was 2,333 times above the laboratory colony and 152 times above the parent strain. Adults of this strain exposed on panels treated with insecticides showed resistance to DDT >10000 times higher than the regular strain of Anopheles quadrimaculatus but were nonresistant to malathion.

Studies are still in progress to develop new insecticides than can be used as residual sprays where Anopheles mosquitoes have become resistant to DDT and dieldrin. In laboratory tests 6 chemicals have been sufficiently effective to justify further testing in buildings naturally infested with mosquitoes. Tests are being conducted to select insecticides that will be effective as residues on a dacron polyurethane fabric for use in the Army

shelter system. Of the materials evaluated so far, Bay 39007 and diazinon have been the best, and produced 100% mortality of Anopheles quadrimaculatus adults for 24 weeks. Dursban was slightly less effective. Malathion, chlordane, and Mobil MC-A-600 were ineffective after 1 week or less of aging. None of the insecticides appeared to damage the surface of the material.

Cloth strips treated with Bay 39007 and resin strips impregnated with dichlorvos have been tested in tents as residual type fumigants against adult Anopheles quadrimaculatus confined in cages. Bay 39007 was highly effective for 2 days or less at a dosage of 2 g/10 m^3. However, dichlorvos at the same concentration produced more than 80% mortality for 19 to 22 days.

Research was continued at Corvallis, Oreg., on the evaluation of insecticide resistance and the development of insecticides. Resistance to DDT was demonstrated in Culex tarsalis at Oakridge, Oreg., 10 years ago. Although DDT has been used little or not at all for mosquito control there since, the resistance persists. Abate, Dursban, and fenthion are all highly effective against mosquitoes resistant to DDT and malathion in a laboratory strain of Culex p. pipiens. Larvae of Mansonia perturbans showed high tolerance to malathion and DDT and a surprising amount of tolerance to fenthion, with some evident even to Dursban and Abate. These appear to be natural tolerances.

Abate and Dursban are two of the most promising mosquito larvicides. Last year's tests gave little residual larvicidal action at the low dosages tested; slightly higher dosages (0.06 lb Abate per acre and 0.035 lb Dursban per acre) gave averages of 13 days and 18 days protection, respectively for the two materials.

Eighty-four compounds were evaluated as repellents and toxicants for wild Aedes dorsalis mosquitoes in spot tests on cattle. Three of the compounds which had shown promise in 1965 again showed good repellency for several days.

About 3 dozen potential systemic insecticides were tested. In tests with 6 insecticides given orally to cattle, all caused some mortality of Aedes aegypti feeding on the treated cattle. The best were trichlorfon and famphur, which gave 98 and 100 percent kill, respectively, at the highest dosage tested. Another finding in these tests was that stable flies were more susceptible to these materials than mosquitoes. Several compounds ineffective against mosquitoes also killed stable flies.

In research under contract with the University of California to evaluate promising insecticides for mosquito control in low-volume applications, promising results were obtained with fenthion and Dursban. Mosquito control in pastures was usually 100%, but results were less effective in dense rice fields until the airplane nozzles were pointed forward which reduced droplet size and apparently increased penetration.

Research was conducted under cooperative agreement with the University of Florida at Gainesville, to study the genetic basis of resistance to chemosterilants by a mosquito. Chemosterilant dosages are being explored as a prelude to genetic tests.

2. House flies - At Gainesville, Fla., the search for new insecticides effective in controlling house flies was continued. In laboratory tests, 5 experimental materials were more effective than the ronnel standard against insecticide susceptible and resistant house flies. Residual tests against house flies in Florida dairy barns were conducted with 9 insecticides. Dimethoate and fenthion were the most effective, but they produced more than 50% control for only 3 to 14 days.

Research was continued at Corvallis, Oreg., on the development of insecticides and the evaluation of insecticide resistance. The resistance spectra of 8 strains of house flies were measured in tests with 13 insecticides (organophosphates, carbamates, chlorinated hydrocarbons, and the botanical product, pyrethrins). Some strains were resistant only to a limited number of insecticides, others were resistant to most of those tested.

In other studies with a parathion-resistant strain of house flies, increased tolerance occurred to phosphates containing O-methyl esters, but lesser tolerance to phosphates containing di-n-propyl or diisopropyl esters. Exposure of malathion-resistant house flies to malathion synergized with DEF (S,S,S-tributyl phosphorotrithioate) resulted in increased resistance. However, the increase in resistance was only 5-fold in 29 generations, suggesting that combinations of organophosphates and synergists or use of O,O-dialkyl phosphates show promise for control of flies resistant to standard phosphate insecticides.

Commercial 20% dichlorvos resin strips were found to be highly effective against larvae of flies breeding in sewage pits at a slaughter house. Mortality of larvae was complete within 5 days and some reduction in fly breeding continued another week, even after removal of the dichlorvos resin strips.

At Beltsville, Md., investigations of physical methods for fly control were continued in cooperation with the Animal Husbandry and Agricultural Engineering Research Divisions. Research on the effectiveness of farmstead sanitation confirmed previous findings that significant house fly reductions (about 33%) can be achieved by sanitary measures on a single farm when unsanitary farms are so close as 1/2 mile. Flies dispersed primarily upwind and to "dirty" areas with many potential breeding sites, rather than to "clean" areas.

Research was conducted under cooperative agreement with the University of Florida at Gainesville, Fla. to study house fly insecticide resistance.

Continuous exposure was found to be the simplest method of determining resistance in house flies.

3. Stable Fly

Research continued under contract with the Florida State Board of Health at Panama Beach, Fla., on control of dog flies (stable flies). Naled continues to look promising as an adulticide, but DDT is the most effective larvicide applied to beach grasses breeding these pests. Bay 39007 appears to be about as effective as DDT for this use. DDT is relatively ineffective against adult flies.

4. Cockroaches

At Gainesville, Fla., research was continued on the development of new and more effective insecticides for cockroach control. In laboratory tests with contact sprays against male German cockroaches, which are resistant to chlordane, 21 compounds produced 75 to 100% mortality within 24 hours at a concentration of 2% in deodorized kerosene. These compounds and others were evaluated as residual deposits on plywood panels against male German cockroaches. ENT-27448 was the most effective material, producing 90% kill as a fresh treatment and then 100% mortality for at least 4 weeks. This compound is an organophosphorus insecticide with an acute oral LD-50 to rats >1000 mg/kg.

At the request of the Armed Forces Pest Control Board, dust formulations of insecticides are being developed for cockroach control. The most promising formulations evaluated recently contained Mobil MC-A-600 as the toxicant. Dusts containing 5% Mobil MC-A-600 in pyrophyllite have produced 100% mortality within 24 hours of exposure for a minimum of 20 weeks when treatments were applied to plywood panels at the rate of 500 mg of formulation per ft^2 and male German cockroaches were exposed on them for 30 minutes. A 2% formulation killed all of the cockroaches for only 1 week.

Dursban has been found highly effective as a residual spray containing 0.5% toxicant in tests against natural populations of German cockroaches in buildings. The average reduction obtained ranged from 93% within 1 day to 95% the second week. It remained at 98 to 99% from the third to the twelfth week.

Efforts are being made to develop improved bait formulations that can be used against cockroaches. When German cockroaches fed ad libitum on baits containing 2% toxicant in peanut butter, Bomyl produced 70% mortality in 1 day and 100% within 3 days. An experimental compound, ENT-27154, caused 90% mortality within 3 days and 100% within 8 days. Mirex required 15 days to kill all of the cockroaches. The trichlorfon standard produced 95% mortality within 1 day and 100% mortality within 9 days.

5. Body Lice - Research was continued at Gainesville, Fla., to develop more effective insecticides for the control of human body lice. Forty candidate synergists were tested in combination with two or more of 4 carbamate insecticides, carbaryl, Bay 39007, ENT-25736, and Zectran, against body lice in the laboratory. None of the synergists were highly effective but one (ENT-20644) showed some synergism when combined with each of the 4 insecticides, one (ENT-21116) showed some synergism when combined with 3 of the insecticides, and 7 when combined with 2 of the insecticides.

Forty insecticides found promising in primary screening tests were evaluated in secondary tests. Abate and eight experimental compounds were equal in effectiveness to the standard, malathion. Four compounds were highly effective but less effective than malathion.

Four candidate louse powders containing Mobil MC-A-600 at 2% and 5% and bromophos at 2% and 5% respectively were compared with a standard powder containing malathion at 1% in tests on research subjects at Camp Lejeune, N. C., in cooperation with personnel of the U. S. Naval Medical Field Research Laboratory. All powders were 100% effective after 1 week. In a second series, Bay 37342 at 5% and the standard malathion at 1% were the most effective (98 to 100%) from the 4th through the 6th weeks. In the 4th week both of these powders were significantly more effective than Bay 37342 at 2%. In a third series all of the 5 powders tested were 100% effective through 2 weeks. Mobil MC-A-600 at 5% was effective for 6 weeks and at the end of the 4th week was more effective than the other powders. Averages of the results for the 4th, 5th, and 6th weeks showed the descending order of effectiveness as follows: 5% Mobil MC-A-600 > 2% carbaryl plus 2% piperonyl butoxide and 5% Bay 37342 > 1 % malathion > 2% Bay 37342.

6. Fleas and Mites - Recent tests have shown the DDT and dieldrin impregnated paper in the kit used by the World Health Organization to determine the presence of resistance in populations of the rat flea (Xenopsylla cheopis) continue to be suitable for use 66 months after preparation.

In residue tests against normal rat fleas, experimental compounds ENT-27324, ENT-27334, and ENT-27300 produced at least 90% mortality in laboratory tests throughout a 24-week test period. Union Carbide UC-21149, and experimental compounds ENT 27230, and ENT-27343 maintained this degree of toxicity for 8 to 12 weeks.

Field tests have been conducted against chigger mites to compare the toxicity of fenthion, Bay 39007 and chlordane emulsions and chlordane granules applied at the rate of 2 lb of active insecticide per acre. In tests made on 50 ft x 50 ft plots, Bay 39007 was the outstanding compound producing 97% to 92% control from 24 hours to 3 weeks after treatment.

Fenthion produced above 90% control for less than 1 week, and the chlordane treatments never caused more than 79% reduction.

7. Bed Bugs - At Gainesville, Fla., research was continued on the development of more effective insecticides for bed bug control. Three compounds have been tested that caused 90% mortality in laboratory tests for at least 24 weeks. Two others were effective for 20 weeks.

8. Fire Ants - At Gainesville, Fla., research is being conducted to develop new bait toxicants for use in the control of Solenopsis geminata. Recent studies have shown that neither filtered nor unfiltered citrus seed oil is effective against this species of fire ant.

C. Biological Control

1. Mosquitoes - At Gainesville, Fla., research on pathogens of mosquitoes has been continued. Larvae of Anopheles quadrimaculatus and Aedes aegypti exposed to Nosema spores became heavily infected. The A. quadrimaculatus were more susceptible than A. aegypti to the disease. Primary sites of infection are the fat body, gut, and malphigian tubules. Stempellia magna has been found infecting larvae and adults of Culex restuans. A new species of Stempellia has been found in Culex pilosus. Both species are transmitted transovarially and cause high mortality in larvae. Attempts to transmit them per os to other mosquitoes have failed so far. Thelohania legeri has been found in two Anopheles spp. in Florida and its life history is being studied in the laboratory. Stages of the pathogen have been found in late instar larvae, pupae, and female adults but have not been observed in first instar larvae. Thelohania opacita was found in Culex territans.

Coelomomyces fungi have been found in the larvae and adults of Anopheles crucians. In one test Coelomomyces fed to early first instar larvae of A. quadrimaculatus resulted in heavy infections in several specimens. A fungus of the genus Rubettella was found in the larvae of two Culex spp.

Two types of Mosquito Irridescent Virus have been obtained from larvae of Aedes taeniorhynchus. One caused the larvae to be an irridescent pinkish color, and the other produced a bluish-green color. Both types have been transmitted to the first and second instars of Aedes taeniorhynchus in the laboratory. These infections produced approximately 10% mortality. The adipose tissue appears to be the primary site of infection, and as the disease progresses the fat body cells become hypertrophied and eventually rupture, releasing numerous viral bodies into the haemocoele. The viruses have been purified sufficiently that electron microscope studies have been started on them.

At Corvallis, Oreg., a fermentation product of Bacillus thuringiensis was toxic to larvae of Culex tarsalis, with an LC_{90} of about 40 ppm during the larval development period. Toxicity was expressed as failure to survive and emerge as adults. A major problem in use of pathogens is the

lack of a technique to infect a field population of mosquitoes with the pathogen. Exeystation of Thelohania spores can be induced with exposure to gaseous osmic acid, or exposure to oxygen followed by desiccation over calcium chloride. Osmic acid was found to be toxic to Culiseta incidens larvae. Oxygenation was more effective in spores frozen and held at 4° C for about 8 months than with spores kept at room temperature for the same period.

At Lake Charles, La., research on mosquito pathogens as potential biological control agents continues to receive increasing emphasis. Microsporidian (and other protozoan), bacterial, fungal, viral, and nematode parasites continue to be found in various species of mosquitoes. Colonies of 8 species of mosquitoes have been established and colonies of 7 of these harboring pathogens are being maintained.

Protozoan pathogens have been found in 17 species of mosquitoes and include 3 genera of microsporidia and 1 genus of flagellate. One, the Plistophora in Culiseta inornata, has been maintained in the laboratory through 10 generations of mosquitoes. It is transmitted transovarially.

A bacterium, probably a species of Vibrio, was found in three species of mosquitoes and was transmitted to larvae of two other species of mosquitoes in their food.

Fungal infections were found in 9 species of mosquitoes. Some kinds were readily transmitted to other mosquitoes in the laboratory.

At least two species of viruses have been found infecting mosquitoes, and 4 species of mosquitoes have been found to be hosts. One virus has been maintained in the laboratory through 62 successive generations of mosquitoes. This virus is readily transmitted to another mosquito species and back to its parent species. However, another virus is readily transmitted to its host species, but a second species of mosquito will only become 1 percent infected.

Nematodes were observed in 6 mosquito species. Over 90 percent of some Aedes sollicitans (a predominant salt-marsh mosquito species) populations were found to be infected in nature. The nematode in this mosquito is believed to be Agamomermis culicis, reported early in this century but not fully described. It causes the death of many female mosquitoes, and those that survive are unable to lay eggs in normal numbers.

The fact that all of these mosquito pathogens have been found in the field may cast some doubt on their potential usefulness at this time in controlling mosquitoes naturally present in Gulf Coast marshes. However, they may be a principal factor holding mosquito populations in check in "light mosquito years". Further research may show how to increase the efficiency of the pathogens to prevent "heavy mosquito years".

Research was continued under a contract with the California State Department of Health at Fresno, Calif., to study pathogens affecting mosquitoes. A polyhedral virus was found in a field population of Culex tarsalis. The fungus Beauveria bassiana kills mosquitoes in laboratory tests, but results were disappointing in simulated field tests. Attempts are being made to increase its virulence, or improve the method of infecting mosquitoes. Mosquito infections with Protozoa (Stempellia and Plistophora) are also being studied.

Research was initiated under a grant to the University of California at Riverside, on the use of introduced annual fishes as a means of mosquito control. Early studies with the fish Cynolebias bellottii, C. wolterstorffi, and Nothobranchius guentheri indicate C. bellottii is the most promising, at least for California conditions. Eggs are laid even at temperatures near freezing and withstand 10 weeks buried in mud. Development to maturity requires about three weeks after hatching of the eggs.

Research has continued under contract with McNeese State College at Lake Charles, La., on predators and parasites of mosquito larvae. Collections of arthropods were made and identified in the hope of finding good control agents. Corixid bugs and Cyclops (crustacea) did not appear in laboratory tests to be predaceous, though this is reported in the literature. Three types of native minnows consumed large numbers of mosquito larvae (up to a hundred or more per fish per day).

2. Imported Fire Ant - Research under a P. L. 480 grant was continued in Uruguay on parasites of the imported fire ant. The parasitic ant, Labauchena daguerri, is quite effective in decimating and ultimately eliminating colonies of the imported fire ant, but the major objective of present research, how to spread the parasite ant, has not yet been reached. Progress was made in overcoming the resistance of the imported fire ant to artificial infestation with Labauchena. Two other potential parasites, a mite and a beetle have also been found.

3. House Fly - Research under a P. L. 480 grant was continued in Korea with dung beetles. Several species have been found that are easy to rear, and some progress has been made in learning which species most efficiently renders manure unsuitable for fly breeding.

4. Cockroaches - In laboratory tests 5 compounds were evaluated as possible repellents for German cockroaches at Gainesville, Fla. Two experimental compounds; ENT-28544 and ENT-25845 were the best of the group, but they were ineffective within 7 days.

5. Fleas and Mites - Research was continued at Gainesville, Fla., on the development of clothing treatments for control of fleas and mites. Pen tests were conducted to determine the effectiveness of five candidate repellents and the standard M-1960 as clothing repellents against oriental

rat fleas. None of the compounds compared favorably with M-1960, which gave 100% protection for 4 days and 80% protection for 16 days.

Field tests were conducted in Florida to determine the protection from chiggers that might be obtained by sitting or sleeping on treated nets spread on the ground. The nets were treated with deet or M-1960 at 0.5 g/g of netting. When the nets were spread directly on the ground, and hands were placed on the nets, chiggers crawled up the hands between the meshes of the nets, but when a piece of cotton cloth (to simulate, for example, a blanket) was spread on the ground and the net was spread over the cloth the chiggers did not cross the net to reach the hand.

Tests were conducted with mites exposed on trousers treated with lindane at 0.2 g/ft^2 or carbaryl at 1.0 g/ft^2. The mites, Leptotrombidium akamushi, were collected in small numbers from a grassy area on Khoa Yai mountain near Pak Chong, Thailand, and exposed on the trousers within a small cell. All mites were knocked down within 30 sec on lindane-treated trousers that had been rinsed 5 hours. The carbaryl-treated trousers that were rinsed 5 hours allowed the mites to crawl for 15 min, but the ones rinsed for 4 hours knocked down the mites in 10 min.

6. Yellow jackets - At Corvallis, Oreg., studies continued with synthetic lures for yellow jackets. This work began in 1962 and a very potent attractant for these vicious wasps, 2,4-hexadienyl butyrate was discovered. Last year's research turned up 7 more compounds even more attractive than that chemical. The lures are completely specific for yellow jackets. Even bees and hornets are not attracted. As little as 1/100th of an ounce of a lure will attract hundreds of yellow jackets. The research is important because the only previous method of attacking these insects was through treatment of their nests with insecticides and the nests are very difficult to locate.

7. Leeches - In the summer of 1966 tests were conducted in the Khoa Yai resort area of Thailand by Gainesville, Fla. personnel in cooperation with military entomologists to evaluate lindane - and carbaryl-treated trousers as protectants for terrestial leeches. Trousers treated with lindane at 0.2 g/ft^2 or carbaryl at 1.0 g/ft^2 were highly effective after 2 hours of rinsing in water but allowed some leeches to crawl on the treated trousers after rinsing for 4 hours. Trousers treated with carbaryl were highly effective after a wash in hot soapy water and a rinse, but the lindane-treated trousers were only partially effective.

D. Insect Sterility and Other New Approaches to Control

1. Mosquitoes - At Gainesville, Fla., research was continued on the evaluation of the sterility principle for control of mosquitoes. Males of Culex pipiens quinquefasciatus exposed as 1-day-old pupae to 7 to 12 k of gamma irradiation were not completely sterilized. Untreated females

mated to these males showed 87 to 99% sterility. Females irradiated with doses of 2 to 6 k laid fewer eggs than normal when mated with untreated males. As the dose increased the egg production decreased. Doses of 7 to 9 k prevented oviposition. Exposure to gamma irradiation reduced the mating competitiveness of the males. Aedes aegypti exposed as third instar larvae failed to pupate after exposure to 4 k, a few pupated after exposure to 3 k but no adults emerged. A few A. aegypti adults emerged after the larvae were exposed to 2 k, but most of them died on the water surface. Adult emergence was good following an exposure to 1 k, but the sterility was low.

A field study has been conducted to determine the effect of the chemosterilant, apholate, in a sugar-water bait on a natural population of Anopheles quadrimaculatus infesting a cow shed. While 78% of the females that fed on the bait were sterile and 67% of them failed to oviposit, there was no clear indication the baits reduced the number of adult mosquitoes in the treated area.

Studies have been conducted to evaluate dust formulations of chemosterilants against mosquitoes. Technical apholate alone gave almost complete sterility of male Culex pipiens quinquefasciatus. Dusts containing 75% apholate on pyrophyllite caused 98% sterility, but dusts of 50, 25, or 10% apholate on pyrophyllite produced less than 90% sterility. The sterility obtained with undiluted apholate was retained for at least 11 days, and the sterilized males were about equally competitive with untreated males in mating with untreated females. Males of Anopheles quadrimaculatus were also successfully sterilized with undiluted apholate, and these males competed favorably with untreated males in mating with untreated females under laboratory conditions. However, sterile males of both species that were reared in the laboratory and released into a normally reproducing population in a large outside cage did not compete favorably with normal males.

Dust formulations of tepa and metepa have been evaluated against C. pipiens quinquefasciatus under laboratory conditions. Tepa produced 100% sterility at 0.5% on pyrophyllite, which was the lowest concentration tested, and metepa gave >99% sterility at the same concentration.

The colony of Aedes aegypti which developed resistance to apholate through laboratory selection is more than 20 times as tolerant to apholate as the regular colony. This strain shows little, if any, cross-resistance to tepa but has a 3- to 4-fold cross resistance to metepa. Resistance to apholate is less in the adults than the larvae.

At. Corvallis, Oreg., research was also continued to evaluate the sterility approach for control of mosquitoes. Tests with a new cobalt-60 source confirmed dosages of radiation previously found effective in sterilizing Culex tarsalis, but the data suggest there is sometimes more than one

mating by some females. Tepa, hempa, and another chemosterilant were tested as possible sterilants for C. tarsalis larvae. Some sterility was obtained by exposure of the larvae resulted in poor survival of the mosquitoes.

Research was conducted under cooperative agreement with the University of Florida at Gainesville, Fla, to study sterilization of a mosquito by irradiation. Irradiation dosage necessary to produce sterility was determined for adult Culex pipiens quinquefasciatus and a sterilizing dose and a lethal dose was determined for the eggs. Dosages tested with larvae showed lethality without sufficient sterility.

Research has continued under P. L. 480 at the University of Cairo in Egypt on the control of mosquitoes by chemosterilants. The dosage of chemosterilant needed to produce sterility has been established for Anopheles pharoensis.

2. House Flies - Research was continued at Gainesville, Fla., on the development of sterilization techniques for house fly control. One thousand and four chemicals were screened for chemosterilant activity, and 80 of these caused complete sterility in adults. Twenty-one compounds produced 99 to 100% sterility in males when the insects were offered a choice of treated and untreated food.

In an attempt to develop a simple method of sterilizing house flies with chemosterilants, pieces of polystyrene foam have been immersed in various concentrations of metepa or tepa and used to cover house fly pupae to a depth of 2 3/4 to 7 inches. These treatments have produced high sterility in the insects that emerged through them. Applications of 5 and 7.5% metepa produced 97 to 100% sterility while 5% tepa caused 100% sterility, but the house flies exposed to 25% metepa died within 2 days after emergence.

Male house flies have been successfully sterilized by confining them in cages with females that were carrying pads impregnated with Olin 53330 on their abdomen or that had been treated directly on the dorsum of the abdomen with Olin 53330, metepa, or tepa. The males were almost completely sterile for 15 days. When the chemicals were applied directly to the abdomen, males exposed to virgin females treated with Olin 53330 or tepa became completely sterile for at least 15 days. Males exposed to virgin females treated with metepa were completely sterile for only 5 days, but sterility remained high for at least 15 days. Metepa and tepa also were excellent sterilants as direct applications to the abdomen of females that had mated previously. Olin 53330 has not been evaluated on mated females.

Triphenyltin acetate has been found an effective chemosterilant in laboratory tests when offered to mixed sexes of house flies at concentrations of 0.01%-0.025% in a dry sugar bait if the insects had a

choice between treated and untreated food. When only treated food was furnished the flies usually were killed before they could lay. In liquid baits, concentrations of the chemosterilant as high as 2.5% to 5% were needed to produce sterility that consistently approached 100% regardless of whether the flies were offered treated food alone or a choice between treated and untreated food.

At Beltsville, Md., investigations of physical methods for fly control were continued in cooperation with the Agricultural Engineering and Animal Husbandry Research Divisions. Mechanical devices, including electrocutor grids, the grids plus lights, and lights plus toxicant (attractant-toxicant devices) all proved ineffective for control of house flies outdoors in cattle pens. Unlighted devices were no less effective than lighted ones, indicating random flights to all units and not attraction. Attractant-toxicant devices of three types tested inside barns killed slightly larger numbers of flies.

Research has continued under P. L. 480 at the University of Cairo, Cairo, Egypt on the control of house flies by chemosterilants. The dosage of chemosterilant needed to produce sterility has been established for Musca domestica vicina.

3. Ticks - At Kerrville, Tex., engorged nymphal Rhipicephalus sanguineus, brown dog ticks, were exposed to gamma radiation of 500, 1000, 2500, and 5000 rads at 1, 5, 11, and 15 days postengorgement. Nymphs exposed to 2500 and 5000 rads at 1 and 5 days postengorgement did not molt to adults. Adults from irradiated nymphs were mated with normal adults, and effects on engorging, egg laying, and hatch of eggs were recorded. A dosage of 500 rads had no effect. At 1000 rads there was some reduction in size of females, size of egg masses, and percent hatch. At 2500 rads, treated females did not lay eggs, but normal females mated to treated males laid a few eggs that hatched. At 5000 rads, treated females did not engorge; a few untreated females mated to treated males engorged and laid some eggs that hatched.

4. Tsetse Flies - Under a PASA agreement with AID, research was continued in Salisbury, Rhodesia in cooperation with the Agricultural Research Council of Central Africa on the feasibility of the sterile male technique for the control of tsetse flies. In chemosterilization trials overall G. morsitans survival was reduced by wind tunnel and contact treatments with tepa and metepa in both the winter and summer seasons, however, the major factor influencing survival was seasonal. Competitiveness, insemination, and sterility were not affected by season. Chemosterilization of 1-day old G. morsitans males with tepa in the wind tunnel and by contact exposure resulted in complete sterility and competitiveness. Competitiveness was less than expected in similar trials with metepa, although complete sterility was obtained.

Wind tunnel application of 0.25 ml of 5% tepa failed to sterilize G. pallidipes completely, but 0.5 ml was effective and provided permanent sterility. Exposure of G. pallidipes males to gamma-irradiation resulted in increasing sterility and decreasing survival as the dosage was increased from 4,000 to 16,000 rads and as pupal age at the time of treatment was decreased, but complete sterility was rarely achieved. G. pallidipes females, with one exception, were completely sterilized with 4,000 to 8,000 rads; survivals were not affected by the treatment. Irradiation of adult males with 8,000 to 16,000 rads was not as effective as pupal treatment and resulted in decreased survival.

Irradiation of G. morsitans males when 1 or 6 days old with 8,000 to 15,000 rads of gamma radiation from Cobalt-60 resulted in 93 to 97% sterility. Females were completely sterilized with 2,000 to 4,000 rads. Both sexes appeared to survive better than the controls. Fractionated dosages of 2,000 X 4, 4,000 X 2, 4,000 X 3 and 5,000 X 3 given one day apart to pupae did not reduce male fertility quite so much as continuous exposure to 8,000, 12,000, and 15,000 rads, respectively. Male longevity was better than with continous exposures, but below the control longevity, and was similar to that expected from a single exposure to 4,000 rads.

An interesting finding was that sterile G. morsitans males can act as vectors of trypanosomiasis when the sterilization treatment precedes the infection in the fly; the data suggest that transmission is reduced when the treatment occurs after the fly is infected.

Attractant investigations in the laboratory have not indicated that G. morsitans females produce a chemical sex attractant, but preliminary bioassays resulted in positive male response to lipid fractions from virgin, mature females. Males appeared to respond and attempt mating only after visual attraction to active females, but generally lost interest rapidly if they failed to copulate almost immediately. Painting the eyes of the male reduced mating, but not feeding, in small cages. Removing the antennae of either sex or the halteres and wings of females did not affect mating under laboratory conditions.

None of the three large cages at the Chirundu station have provided adequate conditions for prolonged G. morsitans survival. Oxen, warthogs and bush-pigs were used as host animals. Extensive modifications to alter the light patterns within one cage have resulted in improved feeding by the flies, but not survival. In an intermediate size cage, which consists primarily of large cement pipes, survival has been extended to 22 days but 50% of the flies died in the first 5 days. Poor survival also occurred in the vertical cage which encloses two trees. Fly behavior and movement was observed in this cage with the aid of radioactive Tantalum.

Small cages containing G. morsitans were placed inside the large field cages during the trials. Survival was much better within the small cages

than in the large volume of the big cages. Small cages of flies in the controlled environment room and in the varying climate of the wicker cage have demonstrated good survival and reproduction rates, although flies released in the large volume of these two cages do not survive well.

Attempts to concentrate a breeding focus of Glossina have not been successful. After two separate one-year trials, involving the positioning of cattle in favored tsetse areas, no permanent fly buildup has resulted.

Population surveys showed that G. morsitans density on Long Island, the site selected for a small field release experiment, remained relatively stable from November to May, increased rapidly through August-September, and then fell sharply to return to the November levels. Population densities were estimated at 3700 to 5300 per square mile depending on the season. Separate field trials demonstrated that marked G. morsitans males dispersed sufficiently to satisfy the assumptions of the statistical model used to estimate the population on Long Island.

E. Attractants and Repellents

1. Mosquitoes and Gnats - At Gainesville, Fla., the screening of new compounds and competitive testing of promising compounds as personal-use repellents has been continued. A commercial repellent which contained 15% deet and 79% red veterinary petrolatum (used as a sun screen) and a camouflage face paint which contained 25% deet were compared with ethanol solutions of deet as skin repellents. The placebo base of the commercial repellent and of the face paint were ineffective as repellents. The results of the protection-time tests and the rinse-resistance tests showed that the ethanol solutions and experimental formulations were about equal in effectiveness. In wipe-resistance tests the ethanol solutions were significantly more effective than the experimental formulations - the formulation with red veterinary petrolatum resisted 19 wipes and the camouflage paint formulation resisted 20 wipes; whereas, the ethanol solutions resisted 42 and 48 wipes, respectively.

The Aedes aegypti eradication program being conducted by the U. S. Public Health Services has required the development of a security area for studies with this insect. The necessary changes have been made in six laboratory rooms. Research has continued on the suitability of other species of mosquitoes or strains of A. aegypti with genetic markers as possible replacements for regular Gainesville strain. Several attempts have been made without success to colonize Culex salinarius Coquillett from eggs collected in the vicinity of Gainesville.

Observations in earlier studies suggested that human hands leave a residue that is attractive to female A. aegypti on handled surfaces. To determine the attractiveness of the presumed residue and the length of time the attractiveness persisted, a total of 16 tests were made in two series,

2 weeks apart. In each test, a fresh plastic glove was worn on the right hand of the test subject for 1 hour, then turned inside out and tested immediately and at 1-,2-, and 3-hour intervals. The worn gloves attracted an average of 27% of the mosquitoes when tested without aging and 18, 16, and 7% after aging 1, 2 and 3 hours, respectively.

Olfactometer tests showed that a broth culture of bacteria (Bacillus cereus) which was started with smears taken from a human arm was attractive to female A. aegypti mosquitoes. In tests in which carbon dioxide was introduced into the olfactometer concurrently with nitrogen gas which had been bubbled through the culture, a culture which had been incubated for 120 hours was more attractive (significant at the 0.01 level) than cultures incubated for shorter or longer periods. Without carbon dioxide a 96-hour culture was the most attractive but was not significantly different from the 72-hour or 120-hour cultures. When it was compared with the 144-hour culture the difference was significant, and it was highly significant with 24- and 48-hour cultures. The 24-hour culture was significantly less attractive than the 144-hour culture. Bacteria counts showed a general decline in population after 48 hours and up to 120 hours, while at the same time attraction response was on a gradual increase from 24 to 120 hours. In all instances, when carbon dioxide was introduced in the tests the response was greater.

One Hundred and forty-five chemicals were screened as mosquito attractants. Of these, 9 showed some promise as possible attractants. Four of the 9 were propanediols. The technique was revised to include a flow of carbon dioxide (5-10 ml/min) introduced with the candidate chemical to determine whether the addition of the gas would increase the attractancy. Nineteen of 64 chemicals tested with this technique showed some attraction. Five percent or more female A. aegypti were attracted by 4 of these compounds with or without carbon dioxide, by 3 without carbon dioxide and by 12 with carbon dioxide.

Seven hundred and nine chemicals were evaluated for their space repellency when applied to cotton netting (4 mesh) at the rate of 0.5 gram per gram of netting. Ninety-five were effective (permitted passage of less than 10% of the mosquitoes) for 6 days or more. Twelve gave protection of 70 days or more. Four of the 12 were octyl sulfoxides. Field tests were conducted against salt-marsh mosquitoes with 4-mesh netting on booth-like enclosures. Of 14 compounds tested, 5 showed promise. After additional toxicological studies by the U. S. Army Environmental Hygiene Agency are conducted, further field evaluation will be made. Field tests with three space repellents, deet, o-ethoxy-N,N-diethylbenzamide, and the Armed Forces clothing repellent M-1960 on netting, were run at Camp Lejeune, N. C. in cooperation with the Naval Medical Field Research Laboratory. The most effective and practical results were obtained when M-1960-treated nets were used to cover the open ends of tents.

With the cooperation of members of the Walter Reed Army Research Institute and SEATO Medical Research Laboratory field tests were run against Culex p. quinquefasciatus and A. aegypti with bed nets treated with two repellents in Bangkok, Thailand. Bed nets treated with M-1960 and deet gave complete protection from these mosquitoes for more than 15 weeks after treatment. Bed nets treated with the same repellents were tested against Anopheles balabacensis at a camp site south of Korat. The nets were tested only once because of the lack of mosquitoes and results were not conclusive.

Research has continued under a grant to the University of Florida at Gainesville, Fla. with attractants for the eye gnat, Hippelates. Research was undertaken to design effective traps for attractant work, and a score of materials (all natural source materials) tested as attractants. Pastes made from shrimp, shrimp-urea, mullet, and liver-urea were superior to eggs, egg yolks, liver powder, blood plasms, dried blood, urea, egg albumen, hog bile, or two commercial hydrolysate baits.

At Corvallis, Oreg., studies were continued on the attractive principle in log pond waters to mosquitoes. Chloroform extracts of both log pond water and cold-trapped odors from these waters were attractive to ovipositing Culex pipiens pipiens. Even the presence of log pond odors caused more oviposition on distilled water than occurred in the absence of the odors, but more egg rafts were deposited by C. p. quinquefasciatus females when they were able to contact log pond waters. In inital tests, chromatographed fractions of log pond water extracts included fractions highly attractive to ovipositing pipiens, quinquefasciatus, and C. tarsalis, and one fraction was repellent for the three species. However, in further studies with waters from six different log ponds, the repellent fraction was not found with chromatography. There has been some evidence that the presence of cedar was detrimental to mosquito breeding, but no clear-cut evidence of toxicity was found with extracts of cedar roots, heartwood, or sapwood, and waters holding cedar logs received 12 times as many egg rafts of C. p. pipiens as did the distilled water controls. Water from ponds holding Douglas fir logs received 9 times as many egg rafts as the controls. Chloroform extracts of Douglas fir phloem showed little promise, but extracts of bark produced fractions with attractiveness near that of log pond water extracts. The chromatographed Rf values of extracts of fir bark and log pond waters were comparable, therefore further isolation of the fir bark extracts was made. The acid derivatives were more attractive than basic derivatives, but the attractancy was apparently related to a combination of materials and not necessarily to a single extractable.

At Corvallis research continued with what are believed to be sex pheromones of mosquitoes. Virgin female Culex pipiens quinquefasciatus, C. p. pipiens, and C. tarsalis respond to pheromones of the males of their respective species or subspecies, and also to those of the males opposite subspecies or of the other species. To some degree, males are also attracted. Female quinquefasciatus and pipiens given a 6-day opportunity to mate also showed attraction to males of the three species.

2. House Flies - Research was continued at Corvallis, Oreg., on the sex pheromone reported earlier in the house fly. Response of flies was found to be somewhat variable, but the response occurred with wild flies, as well as laboratory strains, and colonization of a wild strain for 12 generations produced no loss or gain of the pheromone. In other tests with individual flies it was noted that apparently the threshhold of pheromone needed to stimulate mating varied with individual flies. A strain of blind flies still responded to the pheromone, although with normal flies a visual stimulus (such as a dead fly or object the size and rough shape of a fly) usually enhances the response. Efforts to purify, characterize, and identify the pheromone are continuing by chemists at Beltsville, Md. Over 250 independent materials, purified fractions, and extracts have been tested, but the pheromone has not yet been isolated. The pheromone is apparently a recognition mechanism, that identifies the opposite sex of its own species for the male house flies, which will initiate strike against any fly their size, including other males, but remain to copulate only with female house flies, which, of course, have the pheromone.

PUBLICATIONS--USDA AND COOPERATIVE PROGRAMS

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AREA NO. 15. BEES AND OTHER POLLINATING INSECTS

Problem. Bees contribute to the production of crops occupying at least 5 million acres. Their pollinating activities perform a necessary service in the production of fruit crops, such as, deciduous fruits, berries, cucumbers, melons, and also for vegetable and legume seed crops which are required for the planting of many hundreds of thousands of acres. This pollinating service is performed incidental to foraging for foods for their own use (nectar and pollen). Honey bees are the most important of all the insect pollinators.

Beekeeping is practiced in all 50 States. This distributes pollinators throughout all the cultivated areas. It makes available a manageable supply of pollinators for use where and when they are required. A self-supporting pollination service for many agricultural crops is provided through the production and sale of honey and beeswax. To maintain an adequate level of pollinators, however, beekeeping must be kept in a profitable condition.

A problem of major significance is the increasing use of pesticides, many of which are hazardous to bees or destroy important pollen and nectar sources. There is need for more knowledge of the management of bee colonies; breeding of improved hybrid bees; physiology and behavior of queens, drones, and workers; and the various diseases and pests of the honey bee and means for their control. There is also need to study the many facets of the complex pollination problem to integrate effectively populations of honey bees and other pollinating insects with crop needs and practices. More knowledge should be obtained about wild insect pollinators and their management. It is also essential to study the effects of farm practices, such as the use of different pesticides, changes in crops, soil management, and harvesting, on the economy of the beekeeping industry and the survival of pollinating insects, and to develop procedures to minimize losses from such practices. Information is needed on nectar and pollen plants for use in conservation program efforts to provide bee forage areas in wastelands, watersheds, and roadsides. The nutrition of bees and the nutritive value of different pollens to bees require intensive investigation together with basic nutrition studies for development of pollen substitutes.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing program involving apiculturists, geneticists, microbiologists, physiologists, and entomologists engaged in basic studies and in research concerned with the application of known principles to the solution of problems in crop pollination by insects for the farmer and problems that affect the beekeeper. Bee breeding investigations at Baton Rouge, La., are cooperative with the State Experiment Stations of Louisiana, Ohio, California, and Wisconsin, and the Ontario Agricultural College, Guelph, Ontario, Canada. Bee management investigations at Madison, Wis., are

cooperative with the Wisconsin and Arizona Experiment Station, the Department of Apiculture at Ontario Agricultural College, Canada, and the Agricultural Engineering Research Division. Investigations on bee diseases are carried on at Beltsville, Md., and Laramie, Wyo., in cooperation with the Louisiana, Wisconsin, and Wyoming Experiment Stations. Honey bee pollination investigations at Tucson, Ariz., are carried on in cooperation with the Experiment Stations of Arizona, California, Louisiana, Utah, and Wisconsin, and the Agricultural Engineering Research Division. Cooperation is also extended to the Plant Pest Control Division in monitoring the effect of pesticides on bees and beekeeping. Wild bee pollination investigations at Logan, Utah, are conducted in cooperation with the Experiment Stations of Arizona, Utah, Louisiana, Wyoming, Idaho, Oregon, Washington, the Crops and Agricultural Engineering Research Divisions, and private beekeepers and farmers.

The Federal scientific effort devoted to apiculture research totals 16 scientific man-years. Of this number 3.5 is devoted to biology and breeding for improvement of the honey bee; 2.0 to management for improvement in productivity of honey bees; 3.0 to etiology of bee diseases and development of control methods for diseases and pests; 4.5 to behavior and utilization of honey bees in the pollination of agricultural and other economic crops; 2.0 to biology and utilization of insects other than honey bees in the pollination of agricultural crops; and 1.0 to effect of pesticides and farm practices on honey bees and other pollinating insects.

In addition Federal support of research is provided under a grant to Ohio State University for 0.5 man-year for bee disease research, specifically Nosema; a grant to Utah State University for 0.35 man-year for bee gland research; a grant to University of Illinois for 0.3 man-year for bee pheromone studies; a cooperative agreement with the University of Arizona for 0.5 man-year for bee hemolymph studies; and a cooperative agreement with Michigan State University for 0.2 man-year for cucumber pollination studies.

Apiculture research conducted under P.L. 480 grants total 18.75 man-years. Bee breeding research is being conducted at the Central Apicultural College, Warsaw, Poland (5.5 man-years), at the Research Institute of Pomology, Skierniewice, Poland (2.5 man-years), and at the Faculdade de Filosofia, Ciencias e Letras de Rio Claro, Sao Paulo, Brazil (3.0 man-years). Bee disease research is underway at the Government Agriculture College and Research Institute, Ludhiana, Punjab, India (3.0 man-years) and at the Instituto Nazionale di Apicoltura, Bologna, Italy (0.75 man-year). Wild bee pollination research is being conducted at the Faculty of Apiculture, Department of Agricultural Zoology, University of Cairo, Egypt (1.5 man-years) and at the Government Agriculture College and Research Institute, Ludhiana, Punjab, India (2.5 man-years).

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 14.0 professional man-years is devoted to this area of research.

PROGRESS - USDA AND COOPERATIVE PROGRAM

A. Biology and Breeding for Improvement of the Honey Bee

1. Pairing of Honey Bee Queens. At Baton Rouge, La., honey bee queens have been successfully paired by masking the antenal receptors with odorifous substances of aerwick, pure vanilla, and citral. This method reduced the time of adaptation to zero and eliminated all fighting behavior by the queens. Paired adapted queens have also been successfully stored in queen banks.

2. Fat Body Development in Bees. At Baton Rouge a preliminary study has shown that fat body development in winter bees correlates with the amount of brood present in the colony. Gas chromatographic analysis showed significant differences in the quantities of different fatty acids occurring in bees with high fat body and those with low fat body development. Studies are continuing to determine the lipid classes present and the lipid composition of summer bees.

3. Storage of Mated Queens. At Baton Rouge mated queens were stored for periods up to six weeks without attendants. High mortalities occurred in the fifth and sixth weeks of storage, possibly due to nutritional deficiencies. Stored queens which were introduced into colonies appeared to lay viable eggs and in a normal brood pattern. However, further information about nutrition will be required before long term solitary storage will be practical.

4. Increase in Honey Bee Stocks. At Baton Rouge additional breeding stocks of Anitolian, Carniolan, Greek, and Romanian origin were added to the stock storage. These lines offer a larger pool of genetic variability for future breeding and selection.

5. Cotton Pollinating Collecting Bees. At Baton Rouge 25 queens representing 7 different breeding groups were produced and sent to the Tucson, Ariz., laboratory for initial selection of preference for cotton pollen collecting.

6. Alfalfa Pollen Collecting Bees. At Baton Rouge queens were produced and artificially inseminated for tests in the alfalfa pollen collection breeding project as follows: 15 of the high preference lines, 15 of the low preference lines, 25 representing a back cross of the hybrid to the high line and 23 of a back cross of the hybrid to the low line. In addition, 4 high and 4 low preference line queens were sent to the Tucson laboratory for testing for cotton pollen preference.

7. Cotton Flower Visitation Evaluation. At Tucson, Ariz., workers from 15 queens were screened for a propensity to work cotton flowers. Four lines showed promise. Three queens were sent to Baton Rouge, but only one remains. This was the first step in an effort to develop a more efficient cotton-pollinating strain of bees.

8. Chemosterilant Treatment of Sperm. At Tucson preliminary experiments were carried out to determine the effects of a chemosterilant (tepa) on honey bee sperm in vitro and in vivo. The results showed that tepa affects sperm under both conditions but with considerable variation. There were also indications that not all the tepa reacted in sterilization. In addition, there were apparently side effects from tepa in treated sperm that affected the queens as indicated by delays in the onset of egg deposition.

9. Selection of Alfalfa Pollen Collecting Bees (APC). Studies were continued on the alfalfa pollen collecting project designed to produce high and low alfalfa pollen collecting lines of bees with the breeding being done at Baton Rouge, La., and testing at Logan, Utah. The 1966 tests showed that (1) the number of nectar collectors for both lines was very similar. (2) Pollen collection by the high line was about twice that of the low line where pollen sources other than alfalfa were light, but the positions were reversed when sources other than alfalfa were in relatively large amounts. This could be explained by assuming that where non-alfalfa sources are scarce, strains that select alfalfa will be able to collect the most pollen, but where non-alfalfa sources are abundant strains that reject alfalfa will be able to collect the most pollen. (3) When tested on alfalfa bloom the high line averaged 99% alfalfa pollen collection and the low line 53%. When tested where other sources of pollen were available, the high line averaged 54% alfalfa pollen collection and the low line 2% alfalfa pollen collection. Within a locality there was no overlapping between the alfalfa pollen collection of the high and low lines.

10. Diploid Drones. Bee breeding research under P.L. 480 funds at the Central Apicultural College, Warsaw, Poland, (E21-ENT-7) revealed that not only do adult bees eat diploid drone larvae but also normal worker larvae to some degree. The number of worker larvae eaten varies with the season. Efforts were made to determine what factor influenced the eating of the larvae. When young diploid drone larvae were added as second individuals to cells containing normal larvae both larvae were eaten regardless of whether they were alive or dead. Extraction of diploid drone larvae in alcohol did not remove the feeding stimulant. However, extraction in ether or benzene apparently eliminated the stimulant. On this basis, the hypothesis is put forward that the substance causing the eating phenomenon may be a fatty or waxy compound.

11. Mating Stations. Research on bee breeding conducted at the Research Institute of Pomology, Skierniewice, Poland, (E21-ENT-10) indicates that weather conditions influenced the flight of queens more significantly than did the number of drones present at the mating station. However, the number of drones at the mating station did also materially affect the mating activity. The recommendation is made for increasing the number of drones at mating stations and also enlarging the isolation radius of the mating station to prevent or at least diminish the percent of mismatings by vagrant drones.

12. Wild Bees. Research on bee breeding at the Faculdade de Filosofia,

Ciencias e Letras de Rio Claro, Sao Paulo, Brazil, (S3-ENT-1) has contributed basic knowledge of biological interest with stingless bees. The discovery that Apis sperm entered the spermatheca of Melipona queens seems to indicate that a possible chemical attractant common to both species may be responsible for sperm migration to the spermatheca. Such a chemical attractant has been known to exist in plants for a considerable period of time.

B. Management for Improvement in Productivity of Honey Bees

1. Queen Substance and Queenless Colonies. Studies of the effect of queen substance on queenless bees was made during the May blooming period in the apple growing area near Green Bay, Wisc.. These studies included queenless nuclei, queenright nuclei, and queenless nuclei treated with the pheromone. Behavior of the pheromone treated colonies was similar to queenright colonies. This may have practical application for pollination using disposable queenless packages.

2. Two-Queen Colony Management. Studies at Madison, Wisc., of the economics of various methods of colony management including package, two-queen and single queen management, indicate that efficiency in terms of honey production per unit of time with the two-queen system is superior to all other systems. The srping feeding of pollen supplement increased production by one-third for both single queen and two queen methods.

3. Stored Pollen and Nutrition of Bees. At Madison no effect of aging was found on stored pollen fed within one year in the form of pollen supplement cakes. The pollen used in supplements is more valuable for its bee attracting qualities than for its nutritional value and apparently these attractive elements were not changed during this period of time. Pollen older than two years was not as effective.

4. Alfalfa Pollen Collecting Bees. At Madison the alfalfa pollen collecting stock did not forage well for alfalfa pollen. Competing pollen producing plants, predominately red and white clovers, appeared to be too great a factor in this area. These bees also appeared to have undesirable temperament and burr comb building tendencies. Their honey production was less than 50% that of stocks specifically bred for honey production.

5. Removal of Bees From Honey Supers. At Madison high volume, low pressure air blasts was highly effective in removing supers in one half to one minute with fewer bees remaining in them than by any other method. Modification of equipment using a high duty air compressor with a venturi nozzle to obtain greater air volume was most successfully used for blowing bees.

6. Longevity of Adult Bees. At Beltsville, Md., when 100% cranberry pollen was fed in candy to caged adult bees, 50% of the population of the adult bees died in about 28 days. When the concentration of cranberry pollen was decreased to 50% the longevity of 50% of the population was increased to 31

days. When the cranberry pollen was reduced to 10% the longevity of the bees increased to 42 days. This decrease in longevity associated with increased concentration of cranberry pollen suggests the importance of quality of pollen in wintering bees.

7. Large, Square, Precisely-spaced Combs. At Tucson, Ariz., bees were established in a hive with steel frames 18-3/4" square and spaced precisely 1-3/8" center to center. Over 90,000 bees and a queen were placed in the hive which contained 14 frames, equal to 3.8 Langstroth hive bodies. Excellent combs were drawn; the brood-nest was spherical in shape, the bees built no communication holes, and the frames have been rotated 90° and 180° a number of times with interesting results.

8. Effect of Photoperiod on Honey Bees Studied in a Controlled Environment.

Studies conducted at Logan, Utah, indicated that broodrearing tended to increase following light period increases and to decrease following light period decreases but only after a lag of several weeks. As day length decreased, flight intensity increased and vice versa. Under moderate day conditions, there were two distinct flight periods, mid-morning and mid-afternoon. Under short day conditions, these fused to a single intense flight. On long days, the flight was generally light without distinct peaks.

9. Queen Substance and Behavior of Honey Bees. Research was initiated under a grant to the University of Illinois on a behavioral study of the effect of hormonal secretions of the queen honey bee on the industriousness of worker honey bees.

C. Etiology of Bee Diseases and Development of Control Methods for Diseases and Pests

1. Nosema Disease. At Beltsville, Md., bees infected with spores of Nosema apis differed in their ability to utilize pollen candy diet. The average respiratory quotient of these bees was 1.03, whereas the bees without infection had respiratory quotients which averaged 0.89.

A survey made at Baton Rouge, La., to determine the incidence of Nosema disease caused by Nosema apis Zander was continued. The degree of infection based on the presence of Nosema spores ranged from 0 on March 7 to 26% on April 7 to 14% on April 28 to 25% on May 9 and then declined steadily to 0 on July 27.

At Baton Rouge colonies of honey bees that were opened and manipulated at regular intervals had a significantly higher incidence of Nosema disease than those that were not manipulated. Queen rearing colonies that must be manipulated frequently are likely to have more Nosema disease than those used for honey production.

Nosema apis antiserum was prepared from rabbits inoculated with Nosema spores

at Baton Rouge. This serum was conjugated with fluorescein isothiocyanate and used to stain gut smears from Nosema infected worker honey bees. The antiserum was found to be not specific for Nosema apis. Therefore, a purified antigenic preparation has been made for further antiserum production studies.

At Baton Rouge confined colonies in either the flight room or an outdoor cage and supplied with adequate food showed significantly higher levels of Nosema than free flying colonies. These results indicate that Nosema may be influenced by stress conditions.

At Baton Rouge experiments correlating the Nosema spore levels in feces and those in the mid-gut of worker honey bees were made. The significant correlation obtained indicated that fecal examination provides a reliable means of determining levels of Nosema infection.

At Madison, Wisc., late fall feeding of sugar sirup containing 100 milligrams of fumagillin per gallon fed at the rate of 1 gallon per colony was found to be valuable in delaying the build-up of Nosema in late winter and early spring. These results confirm the findings of previous years.

At Madison fumagillin for Nosema disease control fed as Fumidil-B in sirup had a direct effect upon the life span of caged honey bees when this sirup was their only source of food. However, dilution of medication in the natural food of a colony in the field greatly off-sets this effect.

At Madison a study of Nosema inoculated colonies with or without normal brood emergence and no drug treatment indicated that brood emergence is the prime factor in recovery of colonies from Nosema disease without fumagillin.

At Laramie, Wyo., feeding fumagillin to bees already infected with Nosema apis spores enabled individually inoculated bees to recover.

2. American Foulbrood. Equipment from American foulbrood diseased colonies in Arizona was decontaminated by the use of ethylene oxide in September 1966. After four months, 7 of 16 colonies were still disease free. This method of disease control appears to be highly promising.

At Laramie, Wyo., American foulbrood diseased colonies treated with the antibiotic, Tylosin Tartrate, by gorging with a dosage of 2 grams per gallon of 50% sugar sirup or by dusting with a dosage of 1 gram per 50 grams of powdered sugar cleaned up all visible evidence of this disease after 5 treatments at weekly intervals.

At Beltsville, Md., a continuing survey of the development of drug resistance in samples of American foulbrood received for diagnosis has so far revealed no such resistance to Terramycin in the various isolates of Bacillus larvae made.

At Beltsville decontamination of bee equipment from American foulbrood colonies was accomplished using two introductions of Oxyfume-12® at 8-hour

intervals. No American foulbrood recurred when 2 pounds of ethylene oxide per hive body was used.

3. European Foulbrood. At Laramie, Wyo., gorging European foulbrood diseased colonies with 4 grams gallamycin per gallon of 50% sugar sirup, 3 times at weekly intervals was effective in controlling light infections but failed to control severe infections in the same apiary.

The feeding of various concentrations of albumin and high carbohydrate diets to colonies in New Jersey were ineffective in reducing the incidence of European foulbrood. The elimination of cranberry pollen from colonies was likewise ineffective. Conclusions drawn from these tests and findings of previous years, suggests that the major source of the disease causing organism is contaminated equipment.

At Madison, Wisc., a mixture of one part TM-25 per five parts soy-flour and pollen was developed for European foulbrood control and shown effective. It reduces the threat of antibiotic residue in a surplus honey crop, but does not eliminate its possibility if it is applied to colonies during a honey flow.

At Beltsville, Md., the use of Terramycin or ethylene oxide as a preventive measure for European foulbrood although not completely successful reduced the incidence of the disease significantly. Brood production in treated colonies was much higher than in colonies not receiving the treatments.

European foulbrood is endemic in certain areas of New Jersey. This problem has been especially serious in colonies that are used for pollination of cranberries. This study has revealed that the incidence of European foulbrood is higher before the colonies are moved into cranberries than after one week in cranberries

4. Wax Moth. At Beltsville the present recommendation of 1 pound per thousand cubic feet of ethylene dibromide was found ineffective in killing all stages of the wax moth, Galleria mellonella. Studies indicate that 2 pounds per thousand cubic feet for 24 hours is required for complete control. All tests were conducted at 24° C.

At Beltsville ethylene oxide was found to be effective for the control of wax moth at a level of 23 pounds per thousand cubic feet of 12% ethylene oxide for 24 hours. The tests were conducted at 24° C and all stages of the wax moth were killed.

At Madison, Wisc., preliminary observations indicate that mass application of synthetic sex attractant to a confined area causes virgin female moths to dance to exhaustion. As a result they die without mating.

5. Sacbrood. At Laramie, Wyo., honey bee larvae individually inoculated with more than double the volume of inoculum used in previous tests show that larvae more than three days of age are more resistant to sacbrood disease

than their younger sister larvae.

6. Septicemia. Classification studies on the causative agent of septicemia made at Baton Rouge, La., indicate that this organism would be more appropriately placed in the genus Vibrio than Pseudomonus. Therefore, this organism has been tentatively named Vibrio apisepticus.

7. Ethylene Oxide Toxicity. At Beltsville, Md., levels of ethylene oxide residues less than 1.7 milligrams per milliliter of sugar sirup were not toxic to adult honey bees. However, amounts greater than 6.8 milligrams per milliliter were toxic to bees. The toxicity of ethylene oxide residues in honey was found to be proportional to the amount of water present. Honey with higher water concentrations absorbed more ethylene oxide.

8. Toxicity of Drugs to Adult Honey Bees. At Laramie, Wyo., replicate cages of sister bees in an incubator at 93° F were fed 8 different dosages of various antibiotics in 60% sugar sirup. Terramycin was found to be considerably more toxic than tylosin to adult honey bees.

9. Detection of Drugs in Honey. Progress has been made at Beltsville, Md., on a rapid qualitative detection method for terramycin and sulfathiazole in honey. The method appears to be able to separate the two drugs even if they are mixed together.

10. Dried-Fruit Moth Found Infesting Stored Brood Combs. Larvae of the dried-fruit moth, Vitula edmandsae serratilineela Ragonot has been found infesting stored brood combs at Laramie, Wyo. This is probably a new altitude record (7200 feet) for this species. It has also recently been reported infesting honey combs in several counties in California.

11. Nosema Apis. Research conducted under a grant to Ohio State University indicates that Nosema apis schizogonic forms may be routinely demonstrated in individuals which are free of Nosema spores according to conventional ventricular examination. Nosema spore inoculum from apiculture laboratories in four geographical locations in the United States differ in their infectivity for experimental bees. Intracellular structures, apparently not recognized forms of Nosema found in the ventriculus of honey bees from an Arizona "Nosema-free" area suggest the existence of a new form of Nosema apis or another microsporidian parasite.

12. Hemolymph of the Honey Bee. Research conducted under cooperative agreement at the University of Arizona has shown that no differences exist in the differential hemocyte counts of workers, drones, and queens. Pooled larval hemolymph was hydrolyzed with hydrochloric acid and examined for total amino acid content. In attempts to demonstrate the ability of bee hemolymph to develop immunity, adult bees have been injected with 1% bovine serum

albumin as an antigen. These tests are continuing with total hemocyte counts, differential hemocyte counts biuret determinations, gel diffusion plates, and precipitin titers of immunized and unimmunized bees compared.

13. Mites. Research conducted under P.L. 480 project A7-ENT-10 in India included a survey for acarine infestation of apiaries of Apis indica and wild colonies of Apis dorsata and Apis flora in different regions of India. About 1% of Apis indica, primarily from the Himalayan region, were infested with Acarapis woodi. No colonies of A. dorsata or A. florea were found infested. Infestation was also found in Apis mellifera colonies. Attempts to transmit infestation from A. indica to A. dorsata or A. florea were unsuccessful but transmission from A. indica to A. mellifera were successful. The infestation in Apis indica colonies could be eliminated by 5 to 7 fumigations at weekly intervals with folbex.

In work on acarine disease control, under P.L. 480 project E15-ENT-1 in Italy, further evidence was obtained of the acaricide action of menthol in crystal form in a 10% alcohol solution or blended with vaseline in the proportion of 1:2. Menthol exerts its maximum effect on adult mites within 72 hours, the mortality ranging from 90 to 100%. A technique for laboratory rearing of Acarapis woodi was also developed. This consisted of taking adult mites from the trachea of diseased bees and placing them on the larval integument of mature larvae on parafin beds in an incubator at 28° C and relative humidity about 70%. Pieces of empty trachea of adult bees are also placed on the larvae to provide a place of refuge for the mites. Mites were able to survive under these conditions up to a maximum of 39 days, breeding took place and eggs were laid after 8, 12, 14, and even 19 days and very likely came from matings that occurred on the larval host. Eighty-two eggs were observed from which 22 complete adults emerged. These adults appeared normal even as far as body size and some lived to a maximum of 29 days.

D. Behavior and Utilization of Honey Bees in the Pollination of Agricultural and Other Economic Crops

1. Comparison of Honey Bee Population Estimates in Cucumber Pollination. At Tucson, Ariz., three sampling methods were found to be equally reliable for making comparative measurements of bee activity on cucumber blossoms. Sampling Method A: At each sampling station, ten blossoms within easy sight range were tagged. All ten were constantly observed, and the number of bee visitors during each of three ten-minute periods was recorded for the half hour. Sampling Method B: Ten blossoms were tagged in each of three rows at each sampling station. The number of bee visitations on each ten-blossom row was recorded once during each of three ten-minute periods within a half hour. Sampling Method C: The number of blossoms and the number of bees on or in each blossom were recorded on each of three fifty-foot rows traversed three times within each of three ten-minute counting periods for each sampling station.

2. Survival of Adult Population and Brood Rearing by Honey Bee Colonies Confined in Cages for Pollination Purposes. At Tucson the critical period or the period of adjustment for colonies kept in laboratory flight cages on a pollen substitute did not occur in the first 11 days of confinement. During this period of confinement, the amount of brood either remained constant or slightly increased. The amount of brood dropped in the second 11-day period of confinement when all the original brood was emerging and the population of the colony began to depend on the brood produced. In the third 11-day period there was an upswing in the amount of brood reared which appeared to be related to the surviving original population of the hives. These data suggest that to maintain the original adult population of colonies in confinement in pollinating cages, attention should be paid to the age of bees used.

3. Alfalfa Visitation as Affected by Age of Foragers. At Tucson an experiment established in 1963 was designed to yield information on the amount of separation required to prevent contamination between plots of alfalfa. The plots were arranged to provide gaps of 0, 5, 10, 15, and 20 rods between each plot of a recessive white-flowered strain of alfalfa and a central blue-flowered plot.

Seed harvested at different distances from the edge of each plot were planted and percent of cross-pollination determined by the presence or absence of pigment on the stems of the seedlings.

Two crops have been harvested and evaluated. Both years the plot with no gap between white- and blue-flowered alfalfa showed much greater contamination than the other plots. In all plots, contamination from blue-flowered plants unexpectedly appearing amidst the white tended to obscure the results sought. A gradient in cross-pollination was, nevertheless, clearly discernible as the distance increased from the edge of each plot nearest the blue plot.

4. The Effect of Various Ratios of Female:Pollen-Parent Rows on Crossing Between Several Lines of Safflower. At Tucson a rather complicated plot design was utilized to study the effect on cross-pollination of various ratios of "female" to "male" rows of safflower plants.

Crossing data will not be available until after the seeds have been planted and evaluated on the basis of flower color.

Limited observations on bee activity showed the restriction of pollen collectors to the "male" rows furnishing pollen. Nectar-sugar concentration showed no difference between "sexes", so it becomes clear that pollen distribution will depend on nectar collectors which will visit either line equally. In this situation, pollen collectors may very well impede crossing by removing pollen before it can be distributed.

5. Cranberry Pollination. At Red Lion, N. J., honey bee colonies used for the pollination of cranberries collected more cranberry pollen when

placed in bogs early in the blooming season. Colonies brought in at the onset of peak bloom as a group had a lower percentage of cranberry pollen collection.

6. Pollination of Hybrid Cucumbers. A cooperative agreement was effected this year with Michigan State University to undertake studies to isolate, identify, and evaluate the factors responsible for the most efficient use of honey bees and wild bees for the pollination of hybrid cucumbers.

E. Effect of Pesticides, Insect Diseases, and Farm Practices on Honey Bees and other Pollinating Insects

1. Pesticides and Bees. At Laramie, Wyo., replicated small cage tests with bees exposed to residual insecticides on filter paper showed the approximate median lethal dosages after 72 hours of 10 micrograms per bee for DDT, 4.8 for malathion, 0.4 for dieldrin, and 0.16 for parathion.

F. Biology and Utilization of Insects other than Honey Bees in the Pollination of Agricultural Crops

1. Competition between Honey Bees and Wild Bees on Alfalfa Fields. At Logan, Utah, in tests conducted in blossoming alfalfa fields, honey bees were only weakly attracted to fields with Megachile rotundata, but built up to relatively high levels on fields which had no M. rotundata. The M. rotundata populations seems to be unaffected by the presence of honey bees, but Osmia seclusa was possibly less attracted to the field in the presence of honey bees. Halictus cooleyi was found to gather much of its pollen from tripped flowers and hence was most attracted to the field when M. rotundata was most abundant. Conclusions were drawn that honey bees had no effect on M. rotundata populations, but that M. rotundata made the field unattractive for reentry by the honey bees. Apparently when honey bees are already established on the field, M. rotundata does not drive them out as effectively as it keeps them out when colonies are brought in after M. rotundata are established. Other tests indicated a moderate lowering of attractiveness of the alfalfa bloom to alkali bees when honey bee colonies were present. The alkali bees seem to have no effect on reentry into the field by the honey bees.

2. Green House Propagation of Wild Bees. At Logan, Utah, thirty one species of bees were induced to nest in various materials provided. Soil nesting species generally preferred blocks of sandy loam soil partially buried in gravel used as a water reservoir and placed on a bench rather than the greenhouse floor. Five gallon cans opened on the side filled with sandy loam soil placed in a slanting position on the greenhouse bench were also attractive. Stems of various kinds and drilled logs were also successful in varying degrees to bees that normally use pre-formed cavities.

3. Honey Bee Glands. Research conducted under a grant to Utah State University has resulted in the development of a technique for pipetting the mandibular gland secretion of both worker and queen without any visible harm

to the bees. Two previously undescribed glands have been found in the queen honey bee, the hypopharyngeal gland and the 8th sternite gland. Histological studies of these glands are in progress. Two new glands, the 6th and 7th sternite glands have been discovered in studies with Nomia melanderi, N. triangulifera and Emphoropsis pallida.

4. Other Species of Apis as Pollinators. Research conducted under P.L. 480 project A7-ENT-19 in India has resulted in an extensive survey of insect pollinators of various crops throughout the state of Punjab. The information obtained on Ceratina binghami was of special interest. This insect apparently trips alfalfa flowers efficiently. Its nesting habits are such that it could probably be managed successfully and would be an interesting species to work with in the United States. Work with transporting adult bees indicated that Ceratina survived very well. They have also been found to live for a long time in the field which should make them favorable for management.

Research conducted under P.L. 480 project F4-ENT-4 in Egypt indicates that Andrena ovatula is an important pollinator on broad beans. It is also an important pollinator of alfalfa in Europe, and Southwestern Asia, and of red clover in France. Megachile (Chalicodema) flavipes appears to be a potentially useful pollinator for semiarid regions such as the southwestern United States. It nests gregariously, does not cut leaves, and apparently visits both clover and alfalfa quite freely.

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AREA NO. 16. ANALYSIS, SYNTHESIS, FORMULATION, AND EVALUATION OF INSECT CONTROL CHEMICALS

Problem. Modern insecticides provide a rapid and effective means of controlling injurious insects and their use has enabled the American farmer to produce an abundance of high quality crops and livestock. This extensive use, however, has been accompanied by increasing resistance of some insects to certain insecticides and by the possibility of leaving harmful residues on or in harvested crops, in meat, or in dairy and poultry products. There is therefore a need for the development of new types of chemicals, from natural sources and through synthesis, to which insects will not become resistant. These chemicals should be safe to handle and not leave harmful residues in products used for foods or feeds, or adversely affect wildlife, beneficial insects, and other desirable organisms. More effective and safer formulations of chemicals should be developed for the control of different insect species under various environmental conditions. Such chemicals and formulations require initial testing in the laboratory and evaluation under field conditions before they can be recommended for practical use. It is essential that accurate, sensitive analytical methods be developed for the determination of the amounts of chemicals deposited and the rate of disappearance of their residues and breakdown products in treated crops, animals, or soils. Better attractants as well as lures for additional important insect pests are needed for use in traps and bait sprays for both insect detection and control. Research also is needed on repellents that would be useful in reducing insect attacks on crops, livestock, and man. Insect chemosterilants appear promising for use in insect control and their potentialities should be thoroughly explored.

USDA AND COOPERATIVE PROGRAM

The Department has a long-term program of basic and applied research involving chemists, biochemists, entomologists, and scientists of other specialized disciplines to discover, evaluate, and develop new and improved types of insect control chemicals and methods of utilizing them. Chemical research to discover, isolate, and identify products of natural origin which can be employed for insect control is carried on mainly at Beltsville, Md. Components of the cotton plant that act as attractants, arrestants, feeding stimulants, essential nutrients, or otherwise affect the boll weevil are being investigated at State College, Miss., in cooperation with the Mississippi Agricultural Experiment Station. An investigation of the natural sex attractant of the codling moth is in progress at Yakima, Wash., and an attempt to collect a natural screw-worm attractant is underway at Kerrville, Tex. Grants have been made to the University of Michigan for research on the sex attractant of the tobacco budworm and to Howard University for the synthesis of certain compounds needed in sex attractant studies. A cooperative agreement has been set up with the University of Wisconsin for research on the tobacco

hornworm. A P.L. 480 project has been initiated at the National Botanical Garden, Lucknow, India, to investigate the insecticidal activity of Indian plants. Chemical research on synthetic organic materials and formulations for insect control is carried on at Beltsville, Md.; Gainesville, Fla., State College, Miss., and Yakima, Wash. Contracts have been negotiated with the Midwest Research Institute in Kansas City, Mo., for the synthesis of intermediate compounds needed in the research on insect attractants, repellents and chemosterilants, and with the Hazleton Laboratories, Inc., in Falls Church, Va., for determination of the mammalian toxicity of candidate chemosterilants, attractants, or other new types of insect control agents. A grant has been made to the University of Pennsylvania for the synthesis of compounds wanted for testing as chemosterilants. Development of analytical methods for insecticide residues is carried on at Beltsville, Md.; Tifton, Ga.; Kerrville, Tex.; Yakima, Wash.; and College Station, Tex. There is cooperation with the State Experiment Stations in the respective regions of these laboratories. Cooperative work with the States on insecticide residues is conducted in connection with the following Regional Research Projects: NC-85, Reduction of Hazards Associated with the Presence of Residues of Insecticidal Chemicals in the Environment; NE-36, Pesticide Residues in or on Raw Agricultural Commodities; NE-53, Transformation of Insecticides by Plants; S-22, Agricultural Chemical Residues in Plant and Animal Products; W-45, Pesticide Residues; Their Nature, Distribution, and Persistence in Plants, Animals, and Soils; IR-4, Evaluation of Current Data and Needed Research to Obtain Clearance for Safe, Effective Chemicals for Minor Uses on Agricultural Products. Research on aerosols for insect control is conducted at Beltsville, Md. Biological evaluation of insecticides and other types of insect control chemicals is carried on at Beltsville, Md., and Brownsville, Tex. Research on methods for control of insects in aircraft is done primarily at Beltsville, Md., with a little work during this year at Brownsville, Tex.

The Federal scientific effort devoted to research in this area totals 47.8 scientist man-years. Of this number 11.5 are devoted to products of natural origin as sources of insect control materials; 16.0 to development of synthetic organic materials and formulations for insect control; 9.5 to methods of analysis for insecticide residues; 0.3 to aerosols for insect control; 7.0 to biological evaluation of insect control materials; 0.5 to methods for control of insects in aircraft; and 3.0 to program leadership.

In addition, the Federal support of research under contracts and grants provides 2.5 man-years in this area. Of this total 1.0 is devoted to products of natural origin as sources of insect control materials and 1.5 to development of synthetic organic materials and formulations for insect control.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 35.3 professional man-years is devoted to this area of research.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Products of Natural Origin as Sources of Insect Control Materials

1. Insect Sex Attractants. Chemical research to isolate and identify the natural sex attractants of several important insect species is being done at Beltsville, Md. In the study of propylure (10-propyl-trans-5,9-tridecadien-1-ol acetate), the sex attractant of the pink bollworm, it has been found that the inactive portion of the crude extract from the female moths contains an activator necessary for propylure to attract the male moths in the field. Work to isolate and identify the activator is progressing well. A half pound of propylure was synthesized for use by research entomologists and by the Plant Pest Control Division to detect pink bollworm infestations. Several synthetic homologs of propylure have been shown to be attractive to male pink bollworm moths; these are cis-7-hexadecen-1-ol acetate, trans-7-hexadecen-1-ol acetate, and 10-isopropyl-11-methyl-cis-5,9-dodecadien-1-ol acetate.

Two synthetic analogs of propylure (cis-5, cis-9- and trans-5, cis-9-tridecadien-1-ol acetate) have been found sexually attractive to male fall armyworm moths.

Two procedures have been developed for synthesizing the sex pheromone produced by female fall armyworm moths, cis-9-tetradecen-1-ol acetate. One of these methods should be adaptable to commercial production.

cis-9-tetradecen-1-ol acetate (the fall armyworm sex pheromone) was shown to stimulate male gypsy moths in laboratory bioassay tests. Octadecyl acetate had a similar effect.

The mixed free alcohols obtained by reducing the free acids from an extract of female gypsy moth abdomens have passed stage II in screening against Walker intramuscular tumor at the National Cancer Institute. Beltsville chemists of the Entomology Research Division regularly exchange samples of compounds for screening with the National Cancer Institute.

A purchase description has been prepared for synthetic cabbage looper sex attractant and 0.5 kg of the attractant has been made commercially by a procedure developed by chemists at the Beltsville laboratories. The product was highly attractive to male cabbage looper moths in the field.

The codling moth sex attractant has been isolated; 51 micrograms of the pure attractant was obtained from 300,000 female codling moths at Yakima, Washington. Investigation of the molecular structure of the attractant is in progress there and at Beltsville.

Masking of the effect of the sex attractant of the oak silk moth by substances in the abdomens of the female moths has been demonstrated at Beltsville. An extract made from whole abdomens of virgin female oak silk moths caused a

sexual response in caged male moths. After this extract was ground with abdomens of the moths and filtered, it no longer caused this response. A similar masking effect was previously found with cynthia moths.

In an investigation at State College, Miss. of the sex attractant produced by male boll weevils, a method has been developed for obtaining an active fraction of the attractant by steam distilling mixed male and female weevils. This eliminates the cost of separating the sexes and extracting the males. Active attractant material also has been obtained by steam distillation of feces of the weevils.

At Kerrville, Tex., attempts are under way to collect a natural attractant produced by male screw-worm flies.

At Beltsville work on the extraction and isolation of sex attractants of the sugarbeet wireworm and the oriental fruit moth is in progress in cooperation with the Canadian Department of Agriculture.

Under the grant to the University of Michigan research is being carried on to isolate and identify the tobacco budworm sex attractant. The attractant has been isolated in pure form. A shortage of insects available for this research has retarded progress on the identification.

Under the grant to the University of Wisconsin further progress was made on isolation and purification of the sex attractant of the tobacco hornworm.

Under a research contract the Midwest Research Institute synthesized a number of compounds needed in larger amounts for the sex attractant investigations.

2. Materials of Plant Origin. The constituent of angelica seed oil that is attractive to the Mediterranean fruit fly has been isolated and attempts are in progress at Beltsville to determine its molecular structure.

At State College, Miss., in the study of constituents of cotton squares that are attractive to boll weevils, the compound (+)-1-(1,5-dimethyl-4-hexenyl)-4-methyl-3-cyclohexenol (also known as β-bisabolol) has been isolated and shown to be an important attractant for this insect species. Several other, commercially available, terpene alcohols have been tested and found mildly attractive to the weevils. A second attractive cotton-square constituent appears to be a sesquiterpene alcohol also.

Work was started at State College on the isolation and characterization of components of the gossypol glands of cotton that stimulate feeding by boll weevils. A parallel study with partially refined cotton seed oil was undertaken in an attempt to concentrate material showing strong stimulation of feeding. A compound isolated from the cotton seed oil has infrared and nuclear magnetic resonance spectra similar to a component isolated from the gossypol glands that stimulates feeding. In another study a secondary stimulant component was isolated from cotton and identified as quercetin. In

cooperative research with the Southern Research Institute evidence was found that a very polar compound, probably a polyhydroxy or acidic compound, is an active constituent. In the course of this work 2 flavonoid aglycones not previously reported from cotton were isolated and tentatively identified.

Testing of known compounds and mixtures of them led to formulation of a mixture that is as active as aqueous cotton-bud extracts in stimulating punctures by boll weevils.

A survey has been made of the neutral and polar lipids and the fatty acids in glanded and glandless lines of two varieties of cotton. A correlation with feeding stimulant activity was established.

The flavonoid glycosides in okra (a plant related to cotton) were investigated at State College. Twelve flavonol glycosides and 2 anthocyanins were found and identified. The two major alycones found in okra were quercetin and gossypetin; in cotton quercetin and kaempferol occur.
At Beltsville a cytotoxic alkaloid isolated from Anona glabra was shown to be apparently identical with liriodenine. This alkaloid has shown activity as an anti-tumor agent and is being tested further at the National Cancer Institute.

B. Development of Synthetic Organic Materials and Formulations for Insect Control.

1. Preparation of Synthetic Organic Compounds for Testing as Insect Chemosterilants. A study of the metabolism of the chemosterilant hempa (hexamethylphosphoric triamide) in house flies, using the ^{14}C-labeled compound, was completed at Beltsville. Only one major metabolite, pentamethylphosphoric triamide, was found (See E.2).

^{14}C-labeled hemel (hexamethylmelamine) was synthesized by the reaction of ^{14}C-dimethylamine hydrochloride, cyanuric acid, and trimethylamine. This labeled compound was used in a study of the metabolism of hemel in house flies (See E.2). A parallel study was made of the in vitro oxidation of hemel and served as the basis for identification of the metabolites.

An alkanesulfonate was found to be active as a sterilant for male boll weevils. The synthesis of a series of related compounds has been started to test the effects of various changes in molecular structure on sterilant activity.

Application has been made for a public service patent to cover certain types of organoboron compounds that have a sterilizing effect in house flies or screw-worm flies.

A number of experiments were carried out to develop effective, safe ways of using chemosterilants. At Yakima, Wash., four methods of applying tepa to codling moths were tested to determine which was most convenient and reliable:

aerosol, dipping, residue in glass jars, and microapplication. The aerosol method was most adaptable to treating large numbers of the moths; the residue in glass jars also could probably be adapted to large scale chemosterilization. The persistence of tepa on codling moths treated by the aerosol method at two dosage levels was determined. Dosages of 4.5 and 22.5 micrograms per moth decreased in 72 hours to 0.13 and 2.60 micrograms, respectively; after 144 hours the amount from the 22.5 microgram dose had decreased to 0.56 microgram per moth. At Beltsville the persistence of tepa on treated drosophila was investigated. No tepa was detected on the insects 24 hours after treatment.

As a preliminary to a field trial of tepa in an insect feeder, the persistence of tepa in the aqueous solution and the wick of the feeder was studied at Tifton, Ga. An aqueous sucrose solution containing 0.15% tepa was used. The tepa concentration in the solution decreased by 50% in the first day, then remained constant for the remaining 4 days of the test. The tepa concentration in the wick paralleled that of the solution during the first day, but continued to decrease, reaching about 13% of the original at the end of 3 days. Sodium phosphate buffer radiolabeled with ^{32}P was tested and found useful as an addition to the tepa-sucrose solution to mark the insects that had visited feeders. Insects trapped near a feeder and identified by this means as having visited the feeder were analyzed for tepa. Only 3 out of 12 groups of these insects contained detectable amounts of tepa and none contained more than 4.1 micrograms per insect.

Under a research contract, the Midwest Research Institute synthesized a number of compounds needed at Beltsville as test materials or as intermediates for further synthesis in the chemosterilant investigations.

Chemical studies on the effects and fate of ^{14}C-tepa in boll weevils at State College, Miss., were concluded.

Biochemical studies of the mechanism of resistance to apholate, tepa, and metepa in Aedes aegypti mosquitoes are in progress at Gainesville, Fla.

Under a research contract the Hazleton Laboratories, Inc., have made pilot studies of the dermal toxicity of tepa and hempa to rabbits. Ninety-day chronic toxicity studies are in progress.

2. Preparation of Synthetic Organic Compounds for Testing as Insecticides, Insect Attractants or Repellents, or Synergists. Phenethyl butyrate has been shown to be superior to materials used at present as attractants for the Japanese beetle. A purchase description was prepared at Beltsville for phenethyl butyrate to be procured by the Plant Pest Control Division for use during the summer of 1967.

Of more than 100 compounds of different types that were field tested as attractants for yellow-jackets, the most effective were esters of α,β-unsaturated acids. They were highly specific for yellow jackets, catching no

beneficial species such as honey bees. A good attractant would be useful in protecting working and recreational areas from yellow jackets.

In a search for effective space repellents for protection of people from mosquitoes, hundreds of compounds have been tested by applying them to netting with openings large enough to allow mosquitoes to pass through. The most effective compound found so far is N,N-dipropyl-2-[(p-methoxybenzyl)oxy]acetamide, which prevented for 266 days passage of 90% or more of Aedes aegypti mosquitoes through 4-mesh netting (holes about 0.25 inch square) to a human arm. Several carbanilates, benzamides, and esters gave similar protection for more than 100 days.

A technique has been developed at Gainesville, Fla., for collecting a mosquito attractant material excreted by humans. The attractant is collected by drawing nitrogen through a Plexiglass cylinder in which the person's arm is placed. The nitrogen stream then passes through a train of cold traps where the attractant material is retained.

3. Formulations. At Kerrville, Tex., experiments were started to determine the effects of various organic solvents on ticks and other arthropods attacking livestock. The information obtained will guide the selection of solvents in formulating pesticides for biological tests against livestock pests.

At College Station, Tex., a laboratory method has been developed for the evaluation of formulations of systemic insecticides for application to cotton plants. The results obtained by this method correlate well with biological tests on plants.

Beltsville chemists are cooperating with the Food and Agriculture Organization of the United Nations in developing international specifications for a number of pesticide formulations.

At Gainesville, Fla., a large number of compounds were formulated in various ways for testing against insects affecting man.

C. Methods of Analysis for Insecticide Residues. In cooperation with the Food and Drug Administration, the mass spectra of aldrin, dieldrin, isodrin, endrin, chlordane, heptachlor, heptachlor epoxide, chlordene, and nonachlor were investigated. High resolution mass spectra were determined at Beltsville in this study. The two most widely used techniques for pesticide residue analysis, gas chromatography and thin-layer chromatography, do not yield information on the structure of compounds; mass spectrometry can complement these techniques. The data obtained in this study will be useful in the identification of pesticide compounds, their metabolites, and other reaction products in residues.

Infrared spectral curves have been determined for more than 80 pesticides by an internal reflection technique, and the results compared with the spectra

obtained by the usual absorption technique. The internal reflection method has the advantage of avoiding interferences in analytical determinations due to strong infrared absorption bands from solvents that are used in the usual method.

An automatic spotting device for thin-layer chromatography has been designed and built. This device can be used by unskilled workers in the analysis of pesticides in soils or other types of samples.

In thin-layer chromatographic studies at Beltsville, relative R_F values have been determined for over 40 organophosphorus pesticides on silica gel pH 4 and 7 and acid alumina, with several ternary solvent systems. A transfer technique for a cholinesterase spot test that was successfully applied to the thin-layer plates allows detection of 5 to 6 nanograms (billionths of a gram) of organophosphorus pesticides with P=O groups in the molecule.

At Tifton, Ga., relative retention times and conditions were determined for gas chromatographic analysis of 20 organophosphorus insecticides on 4 different column packings. Temperature programming of gas chromatography has made possible the analysis of compounds of widely different volatilities in a single run with good sensitivity and a large saving in time. The procedure shows promise for monitoring and multicomponent analysis of phosphorus- and sulfur-containing pesticides.

Gas chromatographic procedures using a flame photometric detector for the analysis of residues of several organophosphorus insecticides and their metabolites or degradation products have been developed at Beltsville, Tifton, and Kerrville, Tex. These include methods for coumaphos and its oxygen analog in milk and feces, Shell SD-8447 (2-chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate) and its hydrolysis product in milk and feces, Stauffer R-3828 (S-(p-chloro-α-phenylbenzyl) O,O-diethyl phosphorothioate) and its oxygen analog in animal tissues, malathion and its oxygen analog malaoxon in plants, and diazinon and its oxygen analog diazoxon in corn.

Gas chromatographic procedures for determination of carbamate insecticides and their degradation products, developed at Tifton and at Yakima, Wash., included Mobil MC-A-600 (benzo[b]thien-4-yl methylcarbamate) in silage and milk, Niagara NIA-10242 (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) and its phenolic degradation product in silage and milk, Union Carbide UC-21149 (2-methyl-2-(methylthio)propionaldehyde O-(methylcarbamoyl)oxime), its sulfoxide and sulfone in crops, and carbaryl in apples, potatoes, and other crops.

A rapid procedure was developed at Beltsville for determining microgram quantities of phenols by chloroacetylation followed by gas chromatography with electron capture detector. The method should be applicable to determination of carbamate insecticides after hydrolysis to free the phenols; it also may be useful for determining phenols in water, exhaust gases, smoke, and waste products.

A gas chromatographic method of analysis for the melon fly attractant cue-lure was developed at Beltsville.

A spectrofluorophotometric method was developed at Tifton for the determination of 6-methoxybenzoxazolin-2-one (6-MBOA) in inbred lines of corn differing in resistance to first brood larvae of the European corn borer.

At Beltsville samples of a number of different formulations intended for experimental use in ARS were analyzed to assure that they conformed with their stated content. A sample of a captured Chinese insecticide submitted by the Armed Forces Pest Control Board was identified by infrared and ultraviolet spectroscopy as trichlorfon. A $500,000 lot of malathion emulsifiable concentrate purchased by the Department of Defense and purporting to be made from supergrade malathion was suspected of not meeting specifications because of its odor. Analysis at Beltsville by gas chromatography confirmed by mass spectrometry showed that methyl mercaptan was present in an amount several times in excess of the maximum permitted. Further investigation showed that the process being used by the supplier to prepare supergrade malathion from the technical product was not effective.

D. Aerosols for Insect Control

In tests at Beltsville a cheaper commercial valve than the ones previously approved was found acceptable for the pressurized formulation of deet produced under Federal Specification O-I-503, Insect repellent, clothing and personal application and also for the aerosol formulations described in Federal Specification O-I-507, Insecticide, Allethrin, Aerosol; Insecticide, Pyrethrins, Aerosol. This finding resulted in submittal of new valves by other companies for evaluation and has led to a complete revision of the sections of the specifications on approved nonsealed valves. Six nonsealed valves of the cheaper type have been approved.

Experimental aerosol formulations of a number of new insecticides and synergists were prepared for biological testing at Beltsville (See E.3). Aerosol formulations of the insect chemosterilant tepa were prepared for tests on codling moths (See B.1).

E. Biological Evaluation of Chemicals for Insect Control

1. Insecticides. A major activity in this area is the laboratory testing of synthetic organic compounds and natural products against representative species of insects to determine whether the materials have insecticidal, synergistic, attractant, repellent, or other effects that would be useful for insect control. Preliminary evaluation tests on these materials are carried out at Beltsville, Md., and Brownsville, Tex., by the Pesticide Chemicals Research Branch and at 27 other locations by other Branches of the Entomology Research Division, throughout the United States and in Mexico on 57 insect and 9 mite species. Some of the materials tested originate within the Pesticide Chemicals Research Branch and many others are supplied by other government or

private research agencies and by industry. These materials are also submitted cooperatively for evaluation to the Stored Products Insects Branch, Market Quality Research Division, at Savannah, Ga.

A total of 302 compounds from 46 different industrial or government sources were evaluated in the laboratory for insecticidal or acaricidal activity, 154 compounds from 7 industrial sources for attractancy, and 464 compounds from 39 industrial or government sources for repellency.

One hundred fourteen materials from 30 industrial sources and the Pesticide Chemicals Research Branch were further evaluated in the field. A number of these materials showed considerable promise as broad spectrum insecticides. Mammalian toxicity data, in some cases rather extensive, indicate a good margin of safety in the use of some of these materials.

A new annual compilation listing the colonies of insects, mites and ticks maintained in laboratories of the Entomology Research and Market Quality Research Divisions for pesticide and other research was developed and distributed to government, state, and industrial laboratories conducting entomological research. Two hundred and twelve species are being maintained in 70 laboratories of the Entomology Research Division and 38 in 5 laboratories of the Market Quality Research Division.

2. Materials That Control Insects Through Effects Other Than Death. A study of the metabolism of the insect chemosterilant hempa in male house flies was concluded at Beltsville. ^{14}C-Labeled hempa was used in this study. The only major metabolite of hempa found in the treated flies and in their excreta was pentamethylphosphoric triamide. Unchanged hempa and the metabolite were separated from the extracts of treated flies and excreta by thin-layer chromatography, gas-liquid chromatography, and radiochromatography. The compounds were identified by thin-layer chromatography, confirmed by infrared spectrometry.

The sterilizing potency of hemel hydrochloride by injection into male house flies was determined to be 6.2 micrograms per fly at the 50% effective level. Three hours after injection, 50% of the hemel hydrochloride (applied at rate of 10 micrograms per fly) could be recovered from the flies; about 5% remained in the flies after 24 hours.

The metabolism of hemel in male house flies is being investigated. ^{14}C-Labeled hemel synthesized at Beltsville (See B.1) is used in this study. Four metabolites each have been extracted, isolated, and identified from the flies and their feces. Identification of these metabolites was based on comparison with reference compounds obtained in an *in vitro* study of the oxidation of hemel. Work is continuing on the identification of other metabolites.

3. Aerosols and Space Sprays. (5-Benzyl-3-furyl)methyl (+)cis-trans-= chrysanthemate, developed by British investigators, has been tested in the laboratory at Beltsville in aerosols and sprays. In the spray tests this compound was much more toxic than pyrethrins to house flies and was about equal to pyrethrins in knockdown. In the aerosol tests it appeared to be more toxic to resistant house flies than any other chrysanthemumic acid ester yet developed.

Testing of the chrysanthemumate Neopynamin(R) (2,2-dimethyl-3-(2-methylpro= penyl)-cyclopropanecarboxylic acid ester with N-(hydroxymethyl)-1-cyclohexene-=1,2-dicarboximide) in aerosols and sprays was continued. The compound continues to look promising.

Benathrin (p-allylbenzyl 2,2-dimethyl-3-(2-methylpropenyl)cyclopropanecarboxy= late) at 2% in an aerosol was equal to a similar formulation of pyrethrins in kill of resistant house flies, but was considerably less effective in knockdown. In sprays Benathrin was similar to Neopynamin and allethrin in kill of susceptible house flies, but much less effective in knockdown.

Tropital (bis[2-(2-butoxyethoxy)ethyl] acetal of piperonal) was tested as a synergist for pyrethrins in aerosols and sprays and compared well with piperonyl butoxide. It has been included as a third synergist (in addition to piperonyl butoxide and sulfoxide) in a proposed amendment to the Federal aerosol specification 0-I-507.

Cooperative tests were carried out at Beltsville in connection with development by the Insecticide Scientific Committee of the Chemical Specialties Manufacturers Association of a new aerosol standard for use against the F-58-W strain of resistant house fly to replace the present standard. (The F-58-W strain was developed at Beltsville.) The formula to be adopted for the new standard should give the same knockdown and kill of the F-58-W strain as the present Official Test Aerosol does of the susceptible strain of flies.

F. Methods for Control of Insects in Aircraft.

Entomologists of the Beltsville and Brownsville, Tex., laboratories investigated in laboratory tests the dosages of several aerosols that would be required to kill Mexican fruit flies in airplanes. Complete kill of the flies was given by a dosage of 12g/1000 ft^3 of a 10% allethrin aerosol; 40g/1000 ft^3 of the standard G-1152 aircraft aerosol (1.2% pyrethrins and 3% DDT); or 40g/1000 ft^3 of a 4% Neopynamin aerosol.

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AREA NO. 17. IDENTIFICATION OF INSECTS AND RELATED ARTHROPODS

Problem. Only about a third of the estimated two million or more kinds of insects in the world have been described and named. Many of these are of no known immediate concern to agriculture or mankind, but thousands of species are known to be or are potentially destructive or useful. Minute morphological differences are very important in recognizing many species, and only highly trained specialists are able to positively identify known species and describe new ones. Precise information on the identity and distribution of insects is essential to the efficient conduct of programs concerned with research on harmful insects, the development of methods for their control, and in the management of regulatory activities intended to exclude, control, or eradicate insect pests.

Knowledge of the classification and identification of insects at present is far from adequate. Knowledge of the insect fauna of the world provides the best assurance that any potential pests will be immediately recognized so that appropriate safeguards can be set up to exclude them or prompt action taken to control or eradicate them if accidentally introduced. Moreover, with increasing emphasis on the utilization of beneficial insect parasites and predators to help control destructive insects and weeds, it is necessary that we know which insects to search for, where they might be found, and how to recognize those that may be useful.

USDA AND COOPERATIVE PROGRAM

The program of the Department is a long-continuing one involving insect taxonomists. It includes basic research to make known to science previously unrecognized and undescribed species of insects, ticks, and mites and the application of the results of this research to the problems of insect identification. The work is carried on to a limited extent at Beltsville, Maryland, but mostly at two locations in Washington, D.C., in close cooperation with the U.S. National Museum of the Smithsonian Institution. Cooperation, close but somewhat less active, is maintained with various centers of taxonomic research in the United States and in foreign countries and with numerous individuals in many parts of the world.

The Federal scientific effort devoted to research in this area totals 26.7 scientist man-years. Of this number, 6.0 are devoted to basic studies to name and describe beneficial and injurious insects, mites, and ticks; 11.6 to the identification of insects, mites, and ticks; 7.5 to the preparation of keys and monographs on the classification, distribution, morphology, and biology of insects and related arthropods; and 1.6 to program leadership.

In addition, Federal support of research in this area by means of 5 grants and 2 contracts provides 1.4 professional man-years devoted to basic studies

to name and describe beneficial and injurious insects. One of the grants is to Cornell University for basic studies on the taxonomy, morphology, and ecology of cutworm larvae. Another grant is to Rutgers University and involves basic studies on the morphology of insect sense receptors stimulated by attractants. Two additional grants, both to North Carolina State University at Raleigh, involve basic taxonomic research, one for studies on the nature and taxonomic significance of morphological characters of leafhopper females, the other for studies on the taxonomy and biology of immature stages of the moth genus Acrobasis. A fifth contract, only very recently initiated at the American Entomological Institute, Ann Arbor, Michigan, will investigate the taxonomy and host relations of the parasitic Hymenoptera subfamily Porizontinae. Research on one of the contracts comprises a taxonomic study of the North American weevils related to the boll weevil, and of various populations of the boll weevil itself, by the Agricultural Experiment Station of Texas A&M University. The other contract will result in the compilation of an Index to Fascicle VI, General Catalogue of the Homoptera, now being completed by the North Carolina State University at Raleigh.

Research in this area is also conducted under 11 PL 480 projects. In Uruguay, S9-ENT-6 provides for 1.5 man-years devoted to the classification of grasshoppers; and in Colombia, S5-ENT-2 has 2 man-years devoted to a biochemical study of Drosophila classification. Seven projects are operating in India as follows: A7-ENT-24 provides 3 professional man-years for a systematic study of thrips; A7-ENT-28 provides 2.25 man-years for taxonomic studies of Mallophaga (biting lice); A7-ENT-29 provides 2 man-years for a taxonomic study of Bruchidae (seed weevils); A7-ENT-36 provides 2.0 man-years for a taxonomic survey of encyrtid parasites; A7-ENT-37 provides approximately 2.5 man-years for a taxonomic survey of parasitic Ichneumonidae; A7-ENT-51 provides 1.5 man-years for studies on the systematics of the aphid genus Macrosiphum; and A7-ENT-58 provides 2.5 man-years for studies on leaf-mining Diptera. A project in Egypt, F4-ENT-2, provides 4 professional man-years for a study of the insect fauna of Egypt. In Pakistan, A17-ENT-10 provides 1.5 man-years on leafhopper taxonomy.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 23.1 professional man-years is devoted to this area of research.

PROGRESS - USDA AND COOPERATIVE PROGRAM

A. Basic Studies to Name and Describe Beneficial and Injurious Insects, Mites, and Ticks.

1. Beetles. Three new genera and ten new species of Anobiidae were described during the year in continuing taxonomic studies on that beetle family, and some names of species already known were changed to conform to the rules of nomenclature.

The larvae of Phradonoma tricolor, a dermestid beetle very closely related to and closely resembling the khapra beetle, were described from specimens taken from the hold of a ship containing debris of sesame, cucumin, and peanut shells. The larvae appear to feed on dead insects rather than on stored food products.

In a continuing study on the boll weevil and its relatives, being conducted on contract with the Texas Agricultural Experiment Station, weevils originally collected on wild cotton in Arizona and reared through six generations on artificial media and on cultivated cotton were found to resemble much more closely than earlier specimens the weevil that is normally found on cultivated cotton. Taxonomic revision of the weevil tribe Anthonomini has been about 75% completed. Ecological and biological notes on seven species of the tribe have been made available, and a previously unknown species from Colombia was described.

Forty species of seed weevils have been collected by personnel working on a PL 480 project in India investigating the taxonomy of the family Bruchidae. A parasite which may be of value in the biological control of at least two of the species has also been found.

2. Thrips. Approximately 300 species of thrips have now been recorded from India in studies on that group of insects being conducted by a PL 480 project. Over 1,550 slide-mounted specimens representing 235 species have been received for deposit in the National Insect Collection, and several new genera and many new species have been described as new. Biological studies have confirmed that, of the morphologically indistinguishable species, some are distinctly host specific but some, occupying the same host, are reproductively isolated from each other.

3. Sawflies. Edward Norton was one of the first entomologists to study North American sawflies, but his study collections were dispersed erratically and the species he described have not been restudied in the light of recent taxonomic developments in this group of insects. A recent examination of his material has resulted in important changes in the names of several economically important species. In addition, two sawfly species known to occur in Europe and Japan were discovered also in British Columbia, Ontario, and Quebec for the first time during the year.

4. Moths. Name changes were also made in some economically important moths as the result of recent but continuing taxonomic studies; the parsnip webworm and the potato tuberworm were given new names to reflect better-known taxonomic relationships of each. The type-species of the moth genus Cosmopterix was also discussed in publication.

Among the cutworm moths, a new species of Opsigalea from Texas was described, as were several Neotropical species belonging to the noctuid subfamily Agaristinae.

5. Mealybugs. A mealybug intercepted in Hawaii on Gardenia sp. from the Philippine Islands was submitted recently for identification. It bears a striking resemblance to a native Hawaiian species, and as the two species are distinctly different from any others, they have been placed in a new genus erected for them; the Philippine species was described as new.

6. Two-winged Flies. Recent investigations on range grasses being conducted at New Mexico State University have revealed that a previously undescribed chloropid fly seriously affects the production of seed in range grasses in that State. The same study revealed the presence of another chloropid that is predaceous on grasshopper eggs. Further taxonomic studies on members of this fly family have revealed the presence of three previously undescribed Brazilian species.

For more than 30 years, residents of central and northern Florida have reported annoyance by a Culicoides biting midge. It has proven to be a previously undescribed species closely related to one which is native to Europe and annoys sheep where it is abundant.

A new species of mosquito from Samoa was described, and two flies related to mosquitoes were described from material collected during a Smithsonian-sponsored survey of the island of Dominica, British West Indies.

Personnel working on a PL 480 project in India have begun the collection of leaf-mining flies of that country. Although the project has not been in operation long, it has already accumulated a large number of flies on which biological and taxonomic studies are now being conducted.

7. Wasps. Parasitic wasps of the family Encyrtidae are being surveyed in a PL 480 project in India. Progress reports indicate that an impressive number of specimens have been collected and that six genera have been found to date. Many of the wasps have been reared from known insect hosts.

In another PL 480 project, nearly 30,000 ichneumon flies have been collected in various places in India. This material has been pinned, labeled, and sorted at least to subfamily. Many new genera and species are represented.

8. Leafhoppers. In Pakistan, a PL 480 project has so far resulted in the collection of about 79,000 specimens of typhlocybine leafhoppers from various countries in the Oriental Region. Now sorted into about 750 series, they are being dissected, illustrated, and described.

9. General. The survey of the insects of Egypt has now been completed as a PL 480 project. Approximately 30,000 specimens representing all the insect orders and most of the families known to occur in Egypt were collected and are being identified. A large share of these specimens will become a part of the National Collection of Insects in Washington, and when the identification of this material is complete, taxonomic specialists will

have an invaluable reference collection as a working tool for identification and further investigations of the insects of that area. As a result of direct cooperation by a Branch specialist with Egyptian Ministry of Agriculture personnel, a complete list of the Diptera of Egypt has been compiled.

A private collection of insects numbering about 88,000 specimens has been donated to the National Collection of Insects by A. W. Stelfox, a taxonomist living and working in Dublin, Ireland. Included in this collection were about 70 boxes of identified Braconidae and 10 of Proctotrupoidea, which augments in a very important way the reference collections used by the USDA specialists in these families.

B. Identification of Insects, Mites, and Ticks.

Authoritative identifications and references to pertinent taxonomic and biological literature are supplied to support Federal and State research, extension, control, and regulatory activities pertaining to entomological problems. These services are also performed for industry, pest control operators, and private individuals in the United States and for foreign agencies and institutions concerned with entomology.

During the year, a total of 31,154 lots of insect material was received for identification. About 264,149 specimens were examined. A total of 81,968 identifications was made and reported. Specimens were accepted for identification only when rendering the service could be justified, as there is a backlog of material awaiting study.

The source of material and the numbers of identifications made of the specimens received from each are shown in the following table:

Source	Number of Identifications	Percent of Total
Agricultural Research Service		
Plant Quarantine Division	26,256	32.03
Plant Pest Control Division	1,487	1.91
Entomology Research Division	4,365	5.33
Forest Service	1,157	1.51
Other Federal Agencies	2,733	3.33
States and Insular Possessions	28,789	35.37
U.S. individuals	9,568	11.67
Foreign agencies and individuals	7,253	8.85
Total determinations	81,968	100.00

Many of the specimens received for identification are of much interest, either representing new species not previously in the National Collection or documenting new distributional and/or other data. For these reasons,

49,339 specimens of especial value were added to the National Collection during the year.

The systematic review of technical literature essential to the programs in this area included the examination of 1,589 publications which contained 5,619 articles of interest to insect taxonomists. Reference (by author) cards to these articles totaled 9,174. A total of 2,161 articles was cataloged in depth, and from this effort 29,326 file cards were made up, on which data of significance to taxonomists were recorded. The cards are in continual use in research and service activities, and the file for each specialist is kept up-to-date and immediately available to him.

During the year, 84 visitors obtained aid on taxonomic, nomenclatural, and other problems. The visitors remained for varying periods of time, from an hour or so to several weeks, and came from all parts of the world.

C. Preparation of Keys and Monographs on the Classification, Distribution, Morphology, and Biology of Insects and Related Arthropods.

1. Cockroaches. The identification of some South American cockroaches being used for physiological investigations by the Army Quartermaster revealed the presence of two previously undescribed genera. Taxonomic research on these insects has indicated that they have important phylogenetic connections with certain Old World species.

2. Leafhoppers. A key to the 15 species of the leafhopper genus Graminella known to occur in the United States has been prepared. G. nigrifrons, a vector of corn stunt disease, is found in all major corn growing areas of this country. The several color variants found in nigrifrons are illustrated in a recent publication, and the species are keyed and discussed.

Because the species of the genus Lonatura are primarily grass feeders, they undoubtedly play an economic role in pasture and rangeland areas. A taxonomic revision of this genus resulted in the transfer of five of the previously known species to other genera and the description of a previously unknown species from Arizona.

A catalog of the leafhoppers of the world has been issued in separate fascicles and parts during the past several years. A contract with the North Carolina State University at Raleigh is making possible the eventual publication of an Index for the entire series of the final section, Fascicle VI. Parts 1 to 9 of Fascicle VI have now been card-filed and alphabetized.

By means of a grant also with North Carolina State University at Raleigh, the taxonomic characters of the females of leafhoppers are being investigated. A number of structures have now been studied in many leafhopper genera. A classification based on females of the family Cicadellidae is

being developed which will be compared with that already available for males, and the two systems will be adjusted to more adequately reflect the true relationships of the various taxa.

3. Wasps. Species of the parasitic genus Aprostocetus resemble those of the common Tetrastichus wasps to a marked degree, and the same kinds of morphological characters are used to distinguish them. A recent revision of Aprostocetus keys illustrates and discusses all of the known forms.

Soon to appear in print is a revision of the North American parasites of the genus Anastatus. All of the 16 North American species are primary egg parasites, although some additionally attack cocoons of other parasites. Members of the genus attack eggs of the brown-banded cockroach, preying mantis, gypsy moth, the snowy tree cricket, katydid, and other plant feeders.

A Second Supplement to the Synoptic Catalog of the Hymenoptera of America North of Mexico has been issued to keep pace with the rapid expansion of taxonomic activity in this important order of insects. Under the editorship of U.S. Department of Agriculture specialists, the supplement includes literature published from 1957 through 1963, and in some sections, most of the papers that appeared during 1964. It preserves the format of the original catalog, published in 1951.

4. Moths. Microlepidoptera of the gelechioid genus Batrachaedra and of several closely related genera have recently been analyzed for taxonomic characters, permitting taxonomists to identify, by means of pertinent keys, the various species. The genus Batrachaedra itself contains at least two species of potential economic importance - one which attacks palm flowers, the other the flowers of sisal.

5. Beetles. Many investigations have been made to attempt to distinguish morphologically between populations of the boll weevil found on commercially grown cotton and those found on wild cotton (Thurberia) in Arizona. A large number of pupae of the boll weevil and of the thurberia weevil were studied with the objective of telling the two kinds apart. Differentiating characters were found to vary to such an extent that overlapping between the two subspecies occurs, but the use of a combination of characters will permit one to segregate pupae of these two forms satisfactorily.

Beetles of the tribe Eurymetopinae are commonly intercepted on plants from Mexico, and although the group is not considered to be of major economic importance, a few members occasionally damage crops in the southwestern United States. As previous publications have been unusable in the past, a new key to the genera now makes possible the all-important major breakdowns leading to correct species identification.

6. Aphids. The genus Neophyllaphis is commonly intercepted at U.S. ports of entry, although two of the species are known to occur in the United States.

A recent review, which keys and describes the six species known for the world, will assist Plant Quarantine officials and others to identify these insects quickly.

7. Two-winged Flies. The dipterous family Anthomyiidae contains a number of economically important plant-infesting flies. The subfamily Anthomyiinae, the genera of which have recently been keyed, is especially significant, as most species infest underground roots. The up-to-date key enables workers to identify these flies quickly to genus, heretofore a very awkward process.

The genus Asteromyia contains rather large gall midges that make bright colored galls on plants belonging to the composite tribe Astereae. Although these midges are of little economic importance, they are of interest to the taxonomist because of their host relationships. A recent revision of the genus shows that the 33 previously described forms actually belong to only about 12 species, based on their host preferences and an exhaustive study of their morphology. The sections of the revision on morphology and systematic relationships of Asteromyia form a basis on which other genera of the tribe can be revised.

Insect identification specialists have now contributed 11 manuscripts for publication in a series of catalogs of the South American Diptera being published by the office of the Secretary of Agriculture, Sao Paulo, Brazil. Each of these contributions represents the catalog of one fly family as it is known to occur in the Neotropical Region. This project is especially important in bringing together the very widely dispersed, hard-to-obtain literature of that area of the world.

An extensive list of corrections and additions has been published to rectify the errors appearing in "A Catalog of the Diptera of North America," produced by U.S. Department of Agriculture taxonomists and published in 1965. The list does not include the nearly 800 taxa described and named since the catalog appeared.

8. Sawflies. A revision of the sawfly subfamily Blennocampinae, containing keys to, and illustrations and descriptions of, the adults and known larvae of the 72 presently recognized species, will soon be made available for the use of taxonomists and others working with these economically important insects.

9. Mites. As a result of continuing investigations on the mite family Tydaeidae, investigations on two of the genera, Lorryia and Paralorryia, have been completed. These mites are widespread throughout the world, but they are not found in large numbers and little is known about their biology.

Until recently, our knowledge of the plant-feeding mites has been restricted to those in the United States and Europe. A recent investigation of the Tetranychidae occurring in Mexico, therefore, represents an important

extension of our knowledge, and is the beginning of more exhaustive studies on the mite fauna of Neotropical America.

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AREA NO. 18. FOREIGN EXPLORATION, INTRODUCTION AND EVALUATION OF BIOLOGICAL CONTROL AGENTS

Problem. Many of the most serious insect and weed pests in the United States have been accidentally introduced from foreign countries without the insect enemies that keep them under control in their native homes. Some of the harmful insects so introduced have been effectively controlled by later introduction of their parasites and predators. Foreign exploration for beneficial biological control agents of insects and their subsequent introduction, colonization, and evaluation in this country is now a well established practice in the control of introduced insect pests. The use of imported insects to control introduced noxious weeds, although a more recent practice, has shown much promise. The biological approach to the control of insect and weed pests has great potential. Therefore, further foreign exploration is needed and additional research is necessary on the biology, ecology, nutritional requirements, and the most effective manner of utilizing natural control agents if they are to be used to maximum advantage. There is much concern by the public over the insecticide and other residue problems in foods and by conservationists over the potential hazards of insect-control chemicals to fish and wildlife. More effective use of natural control agents in meeting destructive insect and noxious weed problems could materially contribute to the ultimate objective of overcoming the pesticide residue and other hazard problems associated with the use of chemicals for the control of insects and weeds.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing program on the use of beneficial insects. Basic and applied research is conducted on insect parasites and predators of insect pests and on insects that attack weeds, including foreign explorations for beneficial species and their introduction, liberation, and evaluation in this country. A laboratory is maintained at Gif-sur-Yvette (near Paris), France, for studies on the parasites and predators of agricultural pests that have accidentally been introduced from Europe into the United States. At a station in Rome, Italy, studies are in progress on insects attacking a number of weeds, including thistles, cruciferous weeds, Scotch broom, tansy ragwort, Dalmatian toadflax, Mediterranean sage, and Russian knapweed. Research supported by the U.S. Army Corps of Engineers on insects affecting aquatic weeds, especially alligatorweed and water-hyacinth, has been conducted at the National Agricultural Research Center in Castelar (near Buenos Aires), Argentina. In the United States, a receiving station and laboratory is maintained at Moorestown, New Jersey, where major emphasis is given to receiving, propagating, and transshipping insect parasites to appropriate liberation points. A laboratory for receiving, studying, and liberating insects affecting weeds is located at Albany, California. Studies regarding entomophagous insects are also conducted at Riverside, California. The work at Albany and Riverside is conducted with

the cooperation of the University of California and the California Experiment Stations. A long-range program of basic research involving all aspects of biological control of insect pests is continuing at Columbia, Missouri, conducted in cooperation with the University of Missouri and the Missouri Agricultural Experiment Station. Seven grants and four contracts have been executed that are concerned with the study of insect parasites and predators. The grants are to Washington State University, the University of Missouri, the University of Arkansas, Louisiana State University, the University of Minnesota, Cornell University, and the University of Connecticut. The contracts are with the University of Idaho, the University of Indiana, the University of California at Riverside, and the California Academy of Sciences in San Francisco.

The Federal scientific effort devoted to research in this area totals 23.0 scientist man-years. Of this total, 3.0 are devoted to search for and importation of foreign parasites and predators of insect pests; 2.6 to the search for and importation of foreign insect enemies of weeds; 11.0 to basic biology, physiology, nutrition, and evaluation; 5.4 to receipt, liberation, and establishment of foreign insect enemies of insect pests and weeds; and 1.0 to program leadership.

In addition, Federal support of research in this area conducted under grants and contracts provides for a total of 4.0 scientist man-years. Of this total, 0.9 is devoted to search for and importation of foreign parasites and predators of insect pests; 2.5 to basic biology, physiology, nutrition, and evaluation; and 0.6 to studies of native insects that attack weeds of foreign origin.

Eighteen grants from PL 480 funds providing for 46.5 scientist man-years have been executed for projects directly concerned with the study of insect parasites and predators. Eleven of these projects involve exploration for beneficial species that might be shipped to the United States for trial and release against agricultural pests here.

Grants for four PL 480 projects providing for 10.5 scientist man-years have been executed for studies on the biological control of weeds.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 13.0 professional man-years is devoted to this area of research.

PROGRESS--USDA AND COOPERATIVE PROGRAM

A. Search for and Importation of Foreign Parasites and Predators of Insect Pests.

1. Parasites and Predators. Twenty-one species of parasites or predators of eight kinds of insect pests were collected in Europe for introduction into the United States; 18 species of parasites or predators of 12 kinds of

insect pests were sent to the United States from PL 480 projects in India and Pakistan; and 10 species of parasites and predators of seven kinds of insect pests were received from other sources. All of these beneficial insects, approximately 45,000 specimens in 141 shipments, were received by the Moorestown, N. J., laboratory for screening, testing, and transshipment of living material to liberation points throughout the United States or to Department field stations or State Experiment Stations for further testing and propagation before liberation. Eleven beneficial species were collected locally or reared from laboratory stocks at Moorestown. A total of 251 shipments (almost 62,000 specimens) was made available to State or Federal laboratories in 29 States for release and future evaluation.

2. Citrus, Tobacco, and Vegetable Aphids. Excellent progress is being made on a PL 480 project in Taiwan to search for and study parasites and predators of these important crops. Seventeen coccinellid predators have been found, six of which show high potential for aphid control and are readily propagated in the laboratory. A neuropteroid predator, Chrysopa boninensis, is also effective and has been reared on an artificial diet. Trioxys communis was found to be an efficient parasite of the cotton aphid. A large amount of life history information has been obtained on a wide range of parasitic and predaceous species. A PL 480 project in Pakistan has as its objective a survey of the natural enemies of aphids in that country. Initial investigations have located promising survey sites, and a large number of parasitized aphid species have already been found.

3. European Corn Borer. In a PL 480 program in India, where this insect has been found on corn, it was concluded that the common species of Ostrinia found in the weed Cnicus wallichii is an undescribed species previously misidentified as the European corn borer. Thirteen parasites were recorded from Ostrinia sp. and 10 from Heliothis. A total of 11,253 parasites comprising about 10 species have been shipped to the United States for study and release.

4. Scale Insects on Citrus. A study of citrus scale insects in Israel under sponsorship of the PL 480 program has been yielding significant information on the biology of the parasites and predators of citrus scales. Nine species of predators and 31 species of parasites have been found associated with the California red scale, the chaff scale, the soft brown scale, the Florida wax scale, and the black scale, all injurious in the United States. Phenological and ecological data on these natural enemies have been analyzed and summarized for evaluation of their adaptability to mass rearing in the laboratory.

5. Green Peach Aphid. In a project in Pakistan very recently initiated under the PL 480 program, parasites of the green peach aphid will be surveyed to determine which of them most nearly meet the ecological requirements of habitats occupied by that aphid in the United States. Although the work was not initiated in 1966 until after the peak of maximum aphid activity had passed, a few parasites were reared, and in the Spring of 1967, a stock

of Aphidius matricariae was shipped to the United States. It is hoped that stocks of other important parasites will be available from this activity.

6. Review of Introduced Parasites, Predators, and Pathogens. By means of a contract with the University of California at Riverside, information from all available published and unpublished sources on the transfer of beneficial insects from one geographical area of the world to another is being brought together. To date, literature reviews for most of the chapters of a proposed manuscript have been started or completed, and appropriate information on 300 pages comprising four chapters has been written in final form.

7. Brazilian Plant-Feeding Insects and their Parasites and Predators. By means of a PL 480 grant, a two-volume bibliography of references to Brazilian plant-feeding insects and their parasites and predators has been published. The bibliography is to be the companion to a catalog on the same subject which is in its final stages of preparation.

B. Search for and Importation of Foreign Insect Enemies of Weeds.

1. Alligatorweed Flea Beetle. Surveys have recently been made in some southern States for the purpose of observing and studying the alligatorweed flea beetle and its effect on its host plant in various areas where it had been released previously. This evaluation willlead to a further understanding of the factors affecting the growth of flea beetle populations and host plants. Beetles were also redistributed into a total of 11 new sites in five States.

2. Alligatorweed Thrips. Four shipments comprising 8,000 alligatorweed thrips from Argentina were received at the Albany, Calif., laboratory for quarantine and processing prior to release. Culture units were set up to precondition these insects prior to release by propagating an F_1 generation, and at the present time, the size of the culture is large enough to make releases in the southeastern United States possible in the near future.

3. Dalmatian Toadflax. A two-month field trip to 58 localities in Yugoslavia and Turkey yielded large numbers and a great variety of insects feeding upon Dalmatian toadflax. Six genera of insects were heavily represented in these collections. Extensive biological notes have been made on a moth which lives in close association with Dalmatian toadflax and which may have promise as a potential control agent.

4. Natural Enemies of Eurasian Watermilfoil. A project in Pakistan under the PL 480 program is designed to determine whether any insects exist in that country that could be important in the control of Eurasian watermilfoil, a very serious weed pest in several eastern States. A species of beetle identified as Bagons geniculatus was found to cause severe injury to these plants, which failed to flower normally when the beetle was present. A species of moth also appears to warrant further study, and investigations on the life histories of both these insects are being undertaken. Seven

other insects and two species of snails having some degree of association with milfoil have been found.

5. Enemies of Witchweed and Aquatic Weeds. A PL 480 project in India has disclosed several promising species of insects associated with witchweed, nut grass, and Jussiaea repens. Host-specificity studies are well advanced on one or more insect enemies of each of these weeds, and progress has been made in finding pathogens associated with them.

6. Insects that Attack Thistles in Egypt. A PL 480 project is designed to find and study the biology of insects that attack thistles in Egypt and to determine which are promising candidates for introduction into the United States. During the first year of this project, the geographic distribution of thistles in Egypt was mapped and 32 different species of insects were collected in association with the thistle species under study.

C. Basic Biology, Physiology, Nutrition, and Evaluation.

1. Lygus Bugs. By means of a grant to the University of Connecticut, the presence of host specificity in insects parasitic on Lygus and the role played by host plants in their effectiveness as parasites are being studied. An olfactometer has been constructed to test simultaneously the attractiveness of several plants to parasites of Lygus in the absence and in the presence of Lygus spp. Laboratory and field studies indicate that Erigeron canadensis was the most attractive among alfalfa, clover, sunflower, Chenopodium, honey and water. The role played by parasitic Hymenoptera in regulating populations of species of Lygus is being studied in Poland under the sponsorship of the PL 480 program. To date, Leiophron pallipes is the only important parasite to be discovered in populations of the two most significant Lygus species. Detailed field observations on the parasite have been accumulating.

2. Scale Insects. To determine whether a correlation exists between the occurrence of parasites and predators of scale insects and the kinds of plants attacked by the scales, a PL 480 project has been initiated in West Pakistan. Under this project, Aphytis melinus was found to attack Aspidiosus destructor on all their host plants. Comperiella bifasciata attacked all Aonidiella spp. but was comparatively restricted in its host plant range. Aphytis maculicornis attacked Parlatoria spp. and Eudraspidiotus perniciosus on almost all their host plants. In general, the host plants with the highest density of scale population was found to support the largest complex of parasites. In most cases, total parasitization was shown to be directly proportional to the number of parasite species present.

3. Sugarcane Borers. A PL 480 project has yielded a large amount of information on a number of sugarcane borers at various locations in India and on the incidence of the parasites of the borers. Observations on the feeding habits of adults of Campyloneurus mutator and Xanthopimpla stemmator have been reported, and more detailed information on the distribution and rates of parasitism have been obtained for all of the parasitic species under study.

4. Aphids. Detailed observations on the biology of Coccinella septempunctata, the foremost aphid enemy in India, are being made under PL 480 sponsorship in the vicinity of Hyderabad, India, for the purpose of determining how this beetle may be established successfully in the United States. So far, attempts to rear immature stages or to maintain adults on artificial diets have not met with success, nor have local strains been found despite considerable searching. Investigations are continuing in an attempt to elucidate the factors which prevent its immediate introduction into America.

5. Parasitic Beetles. Research on parasitic carabid beetles is being conducted at the University of Arkansas under a research grant. Disonycha glabrata, a chrysomelid beetle associated with plants of the genus Amaranthus, has proved to be the host of the most promising of these carabids, Lebia analis. A great deal of biological information on analis has been obtained to date.

6. Coconut Rhinoceros Beetle. A PL 480 project in India has yielded a great deal of information about the abundance of this beetle and the biology and abundance of its predators and parasites. Life history data have been obtained and rearing methods devised for several of the predators. Marked reduction of beetle damage has already resulted from the application of integrated control techniques. Three of 20 chemicals tested for attractancy gave positive results.

7. Tachinid Flies. Under a grant to Washington State University field studies on a number of parasitic tachinid flies and their hosts are being made. Tachinids have been reared from more than 17 species of insect hosts. Malaise traps have been operated regularly throughout the season at Friday Harbor and Pullman, two localities representing different environments which should produce flies considerably different than those already caught during the project elsewhere in the State. Considerable numbers of tachinids have been identified, and a taxonomic key to the Tachinidae of the Pacific Northwest has been started. A modern host catalog and bibliography of tachinid flies is being produced under contract at the California Academy of Sciences. To date, more than 943 articles have been cataloged and about 4000 individual rearing records have been entered into the recording system.

8. Insects on Rice. Data from research on rice insects and their parasites, made available by means of a PL 480 grant, indicate that 34 species of parasites occur on paddy borers in West Pakistan. Although this is a large complex, the overall degree of parasitism appears to be low.

9. Insects to Control Alligatorweed. Studies in Argentina are being continued on the biology of the alligatorweed thrips and the phycitid stem borer, both of which are being investigated for introduction into the United States as possible control agents for that weed. Laboratory starvation tests are being conducted on both of these insects, and concurrent laboratory and field observations on the adult behavior of the stem borer and a braconid parasite are being recorded. Host preference studies are being

continued on the alligatorweed flea beetle. The native lepidopteran Pachyzancla bipunctalis occurs in considerable numbers on alligatorweed in the late summer and fall in Louisiana. This insect is the subject of biological investigations in that State to determine its effectiveness as a possible biological control agent for that weed.

10. Waterhyacinth. As a result of research made possible by a grant to the University of Louisiana, two lepidopterans have been found feeding extensively on waterhyacinth in this State. One of these, Arzama densa, infests up to 20% of the plants in the areas under study, and because of this, intensive studies on its life history and seasonal and behavioral characteristics are being made in outdoor water tanks under controlled conditions.

11. Mediterranean Sage. For several years the most important factor preventing the introduction of this insect to control Salvia aethiopis in the United States has been the possibility that it feeds on an alternate host, possibly a beneficial plant, during its aestivation period. As the result of an intensive two-month search in France, the aestivation site of this beetle was finally located among crowns of Graminae in the shade of isolated trees in the infested area. This discovery should bring the introduction of this potentially valuable insect one step closer to reality.

12. Rangeland Weeds. Two new county distribution records of Linaria have been added to a list of range weeds worked out by means of a grant to the University of Idaho. From all weed species being studied, 310 collections of insects were made during the growing season of 1966. Many aspects of the biologies of four insects which have promise as biological control agents were recorded and analyzed.

13. Insect Enemies of Weeds Common to the United States and Pakistan. In Pakistan, a PL 480 project is designed to study intensively some insects previously found closely associated with weeds belonging to the genera Halogeton, Cyperus, Carduus, Cuscuta, and Xanthium. The project is looking into the biologies of a promising leaf-mining fly and three species of moths which are highly specific to Cuscuta spp. Attempts are being made to rear two species of moths that feed on Halogeton. Tests showed that the moth Oecobia verbascalis is host specific to Xanthium and is effective in reducing the seed production of that plant. Host specificity studies have been started on several other species of insects known to be associated with these weeds.

14. Development of Superior Parasite and Predator Strains. A grant to the University of Missouri is making possible the establishment of laboratory colonies to determine whether desirable characteristics of selected aphid parasites and mite predators can be improved and propagated. The literature on the subject is being reviewed and field surveys and collections of promising beneficial insects are being made. Spider mite colonies have been

set up in two environmental chambers where the principal studies will be conducted.

15. Testing of Chemical Stimuli. A number of fatty acids and protein hydrolysates are being tested under a research grant to the University of Minnesota to determine their attractiveness to coccinellid beetles. Field tests indicate that a sugar spray increases predatory insect populations in a corn field but fails to affect significantly a manually placed infestation of European corn borer. Among several chemicals tested in a Wilson olfactometer, geraniol and honey proved attractive to coccinellids but "keptanoic acid" (attractive to honey bees) repelled them.

16. Mass Rearing of Parasites. Larvae of Heliothis armigera and Prodenia litura support a large number of parasitic insects in India. By means of a PL 480 project there, excellent laboratory rearings of both species have been obtained through rigorous sanitation and modern techniques. Methods have now been developed to successfully propagate the tachinid Carcelia and three hymenopterous parasites, one of which has been carried through 17 generations with a production of 62,000 pupae. Continued experimenting with these mass rearing techniques should produce substantial future contributions to the biological control effort.

17. Parasite Resistance to Insecticides. A new PL 480 project in Poland is designed to determine the feasibility of developing a strain of parasite that is resistant to insecticides by rearing the parasite on an insecticde-resistant strain of host insect. Results to date have shown only that the immature stages of Trichogramma evanescens within resistant host eggs are 100 times more resistant to metasystox than are the adults. The project has significance in the eventual application of integrated control systems.

D. Receipt, Liberation, and Establishment of Foreign Insect Enemies of Insect Pests and Weeds.

1. Pea Aphid. A survey of alfalfa fields in Ohio, Indiana, Michigan, Wisconsin, and Minnesota revealed that the introduced parasite Aphidius smithi has displaced A. pulcher as the dominant pea aphid parasite of the Midwest. For reasons not known, A. smithi remains much less numerous in New Jersey and adjacent East Coast States than A. pulcher, despite the fact that the former was originally introduced on the East Coast from India. A PL 480 project has yielded a large amount of biological data on the parasites of a number of aphids that occur in India. A total of 1,000 Aphidius smithi was shipped to the United States where they were used in experiments that demonstrated the absence of any reproductive barrier between populations from Ohio and from Dehra Dun, India. These experiments established that smithi was introduced successfully here in 1957. Living material of other species has also been shipped to the United States, and much valuable information is now available to support a growing program for the biological control of aphids.

2. Alfalfa Weevil. On April 26, 1963, 112 adult Bathyplectus anurus were released near Bowmansville, Penn. This year the species was recovered at a parasitization rate of 6.2% from alfalfa weevil larvae as far as two miles away from the original point of release, where it averaged less than 1%. Parasite populations at the original release sites in Pennsylvania and New Jersey have declined sharply during the past three years for reasons that are as yet poorly understood.

3. Vegetable Weevil. During the years 1943 to 1946, four species of parasites shipped from Uruguay were released in southern California against Listroderes, but no evidence of establishment was ever obtained. Now, however, California larvae of the vegetable weevil have been found parasitized by an ichneumonid identified as Tersilochus n. sp. As two apparently undescribed species of this group were known to be present among those introduced in the 1940's, it seems possible that the recently found species is one of those. Further investigations are being made to determine its origin and possible effectiveness.

4. Imported Cabbageworm. Two Pieris-reared strains of Trichogramma evanescens, one from Poland and one from France, parasitized nearly 100% of a laboratory cabbageworm culture in recent tests. Six days after approximately 2000 eggs parasitized by the Polish stock were released on wild mustard plants in the field, 11% of the cabbageworm egg population was parasitized, a much higher proportion than is to be expected from native parasites alone.

5. Face Fly. During the year 12,500 specimens of Aleochara tristis were released at seven different locations in New Jersey, Pennsylvania, and Maryland. The larval stage is predaceous on face fly pupae, while the adult preys on eggs and larvae of Diptera that normally live in cow chips.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Seach for and Importation of Foreign Insect Enemies of Weeds

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AREA NO. 19. INSECT PATHOLOGY

Problem. Basic investigations on viruses, fungi, bacteria, nematodes and protozoa are needed to fully exploit the use of such microorganisms as an approach to insect control. There is much interest in the use of these natural insect-control agents to overcome the growing concern over chemical residues following the application of insecticides to agricultural crops and livestock, and the increasing resistance of some insects to certain insecticides. The utilization of pathogens to produce diseases in insect populations, and so reduce them and the damage they cause, is an approach that has already shown great promise. Microorganisms that are pathogenic for insects are generally very efficient when used properly. They are specific for their insect hosts and harmless to men and other vertebrates. Basic research is needed for a thorough understanding of insect pathogens, including their growth and nutritional requirements, their resistance to environmental factors, and their mutability and mode of action, both in the laboratory and the field. Such knowledge must be obtained before these organisms can be used effectively in the control of insect pests.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing basic research program on the growth, nutritional requirements and mode of action of viruses, bacteria, and nematodes affecting insects. At the Pioneering Research Laboratory on Insect Pathology at Beltsville, Md., studies are in progress on mutability-insects to diseases, including studies of the effect of the environment on the pathogens. A comprehensive reprint library on insect pathology is being assembled. Collections of all spore formers and viruses known to cause disease in insects are being obtained from world-wide contributors. A service involving the diagnosis of unhealthy insects is now available to Division, State, and University laboratories.

A laboratory for serological investigations to aid in all aspects of the research conducted at Beltsville and an insect tissue culture laboratory to investigate production of insect tissue and to study the invasion, metabolism and production of insect viruses has been established at Beltsville.

The program includes collaborative studies with the Pesticide Chemicals Research Branch on instrumentation for monitoring insect activity, internal temperatures of insects, and effect of gaseous atmosphere on metabolism and development of insects. Collaborative studies are also under way with the Pioneering Research Laboratory on Insect Physiology on the effect of microorganisms on insect sterol requirements. A cooperative project to study the progress of the non-inclusion virus disease of the citrus red mite, through electron microscopy, has been set up with the Fruit and Vegetable Insects Research Branch. A second cooperative study on the serology of this virus is underway in cooperation with the Insect Pathology Research Institute, Sault Ste. Marie, Ontario, Canada.

Contracted research continued with Rosner-Hixson Laboratories for studies of mammalian toxicity and pathogenicity of insect viruses and with Ohio State University, Rutgers University and University of Maryland for research on the production of virus disease of four major insect pests. A contract is in effect with Nutrilite Products Inc. to study the production of viruses pathogenic for two more insects of major economic importance and to investigate the possibility of selecting more virulent viral strains of the nuclear polyhedrosis of the cotton bollworm, Heliothis zea. A contract with Midwest Research Institute covers attempts to propagate an insect virus in bacteria and another contract with Cornell University, Geneva is to determine the relative virulence of two, coexisting viral pathogens of the cabbage looper.

A cooperative project has been conducted with the Fruit and Vegetable Insects Research Branch to study the serology of the non-inclusion virus of citrus red mite, in order to develop a tool for diagnosis of the disease. Cooperative studies on the identification of B. thuringiensis varieties are being carried out with the Institute of Pasteur, Paris, France.

Federal Scientific effort devoted to research in this area totals 10.0 scientist man years. Of this number 4.4 is devoted to virus diseases of insects; 4.6 to bacterial, nematode, protozoan and fungal diseases of insects, and 1 to the discovery and study of new pathogens of insects.

In addition, Federal support for 3.3 professional man-years is devoted to research on virus disease of insects under contracts.

Additional research is in progress under P. L. 480 projects at The

Institute of Plant Protection, Poznan, Poland, (1.5 man-years) and at The Institute Annamalai University, Madras, India (3.0 man-years).

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 15.6 professional man-years is devoted to this area of research.

PROGRESS -- USDA AND COOPERATIVE PROGRAM

VIRAL DISEASES OF INSECTS

1. Inducement of cytoplasmic polyhedrosis by intrahemocoelic injection of the viral particle into the silkworm, Bombyx mori. Cytoplasmic polyhedrosis was induced by intrahemocoelic injection of the freed viral particle into the silkworm, Bombyx mori. Examination of tissues of the infected insects revealed that formation of the typical tetragonal type of polyhedron was restricted to the gut epithelial cells. Thus, the study demonstrated that the basement membrane and circular muscle of the gut do not provide mechanical barriers that protect other tissue from infection. Normally, infection is by per os contact and only the gut epithelium is attacked. It has been speculated that other tissue of the insect's body is unaffected because of the mechanical barriers offered by the gut basement membrane and circular muscle. This theory is now disproven. Determination of the ustrintine factors for the insect cytoplasmic inclusion virus are continuing.

2. Attempts to cultivate insect viruses in avian eggs. Nuclear and cytoplasmic polyhedrosis viruses (intact polyhedra and free viral particles) were injected into turkey eggs to determine if insect viruses increased the rate of partherogenesis in turkeys and if insect viruses would propagate in turkey eggs. In each test, using either intact polyhedra or free viral particles, the results were negative. Although the results were negative in that virus propagation could not be produced in avian eggs, this information is of value as added evidence that insect viruses will not multiply in warm-blooded animal tissues.

3. Nucleic acid type of the blue-green and orange mosquito iridescent viruses (MIV). Qualitative nucleic acid analyses of the blue-green and orange MIV isolated from larvae of Aedes taeniochynchus revealed the presence of DNA in both strains and the absence of RNA. Quantitative estimation showed that DNA constituted 10.5 percent in the blue-green type

and 11.7 percent in the orange type.

4. Survival of insect virus in the gut of mammals. The nuclear polyhedrosis virus from Trichoplusia ni was fed quantitatively to white mice by introducing it directly into the stomach. The mice were retained in special cages designed to collect all feces and urine. At two, three, and four days, all feces were collected, processed and were fed quantitatively to fourth instar T. ni larvae. The animals were sacrificed and contents of the gut from buccal cavity to anus were collected, processed and fed quantitatively to T. ni larvae and a sample of tissues including heart, lung, liver, spleen, kidney and muscle tissue were similarly fed to larvae. It was established that: (1) All virus had been passed from the mammal gut in three days (2) no virus was detected in the animals or organs or tissues (3) the viability of the virus was reduced by 98.5%; only 2.5% of the original virus dose remained viable in the feces four days after forced feeding.

5. Studies of new viruses. Two new viruses isolated from specimens sent to our laboratory from Tifton, Ga. have been examined by light and electron microscopy. The granulosis virus isolated from Plodia interpunctella replicates principally in fat body; tissues; replication was also observed in tracheal and epidermal cell nuclei. Blood cells and Malpighian tubules also appeared to contain virus particles. In Cadra cautella, the virus is a nuclear polyhedral type, with packets of virus rods enclosed in polyhedral protein. Replication occurs in nucleic of tracheal matrix associated with the gut, nervous system (brain, ventral nerve cord), reproductive tissues, and muscles, fat body, epidermal cells and Malpighian tubules. Also a cytoplasmic polyhedral virus was isolated from our rearing stock of T. ni.

6. Histopathological studies. Shadowed preparations, carbon replicas and sections have been prepared from the following viruses: T. ni nuclear and cytoplasmic polyhedra; H. zea nuclear polyhedra; Spodoptera frugiperda nuclear polyhedra and granulosis, Spodoptera exigua nuclear polyhedra; Bombyx mori nuclear and cytoplasmic polyhedra; Estigmene acrea cytoplasmic polyhedra and granulosis and polyhedra isolated from grasshoppers at the Bozeman, Mont. laboratory, Grain and Forage Insects Research Branch.

Tissues which are for light and/or electron microscopic examination from infected and healthy insects are in the process of evaluation. There appear to be differences in susceptibilities of tissues in different insects, e.g., tracheal matrix and fat body. In more extensive studies on infected

tissues of T. ni and C. picta it appears that infection occurs by a random process once the virus rods pass through the basement membrane of the gut, infecting the nearest target tissues first. However many observations have been made of tissues in which early stages of polyhedral formation were occurring simultaneously in susceptible tissues. It generally appears that polyhedral formation can be seen first in tracheal matrix nuclei among the connective tissues surrounding the gut. There is considerable evidence to support the theory that the infective particles or virons are virus rods (probably enclosed in membranes) and are phagocytized by the susceptible cells by a process similar to that described for animal viruses. Many virus rods, naked or enclosed in membranes have been observed between cell membranes, at nuclear membranes, and in the cell cytoplasm of susceptible tissues.

Inactivation of insect viruses by exposure to sunlight. Clean nuclear-polyhedrosis virus of the cabbage looper and the granulosis virus of the salt-marsh caterpillar were exposed outdoors on Millipore filters then fed to larvae. Three-hour exposure in direct sunlight inactivated nearly 100% of the polyhedrosis and granulosis viruses.

7. Electron microscopy-cooperative projects. No less than eighteen separate electron microscope studies were carried out in cooperation with insect pathologists in the Division in industry and with Canadian scientists. Branches in the Division involved are Apiculture, Pesticide Chemicals, Grain and Forage Insects and Fruits and Vegetables Insects Research Branches.

BACTERIAL PATHOGENS OF INSECTS

1. Mode of action of B. thuringiensis B. T. δ endotoxin. The biochemical mode of action of the toxic parasporal crystals produced by Bacillus thuringiensis var. dendrolimus was studied. Extensive investigations into the biochemical mode of action of the toxic parasporal crystals at the cellular level has revealed that the toxic moiety (isoelectric point 4.5) reacts ionically with proteins having isoelectric points in the alkaline range. Precipitation and/or inhibition properties of the toxin were exhibited with glyceraldehyde-3-PO_4 dehydrogenase, trypsin and cytochrome-C, but not with hexokinase, pepsin, and bovine serum albumen, the latter having isoelectric points in the acid range. Since pH of the reaction mixtures were found to be critical for precipitation and/or inhibition. (pH 6.0-8.0) the investigators proposed that the toxic effect on lepidopterous insects was caused by: (1) alteration of net charge on important proteins

involved in cellular glycolysis, Kreb's cycle, and oxidative respiration and (2) subsequent inhibition of enzymes, proteins necessary for maintenance of the gut epithelial cells. Preliminary studies revealed the absence of hyaluronidase, proteinase and phospholipase activity as part of the crystal protein.

2. Identification of B. thuringiensis B. T. β exotoxin. Cultures of Bacillus thuringiensis 996 at maximum sporulation were centrifuged to remove cellular materials. The supernatants were lyophilized and a crude, dried extract was subjected to precipitation with 90% ethanol followed with 95% methanol to concentrate the biologically active material with the precipitate.

Further purification of the precipitates by column chromatography followed by preparative thin layer chromatography enabled us to obtain 5 mg of an active fraction which appeared to be a homogenous substance by several thin layer chromatographic solvent systems. By most of the solvent systems employed the migration characteristcs of the biologically active compound (s) was between the mono- and diphosphate adenine nucleotide reference standards.

The electrophoric mobility at pH 3.5 and 7.4 shows similar results with standards, but were not definitive to indicate the presence of the active material as an oligonucleotide.

The characteristic ultraviolet curve showed a maximum of 258 $m\mu$ in neutral solution and in 0.1 N HCl, and a shift to 262 $m\mu$ in 0.1 N NaOH, which was similar in response to the model adenine nucleotides. The spectra of brominated samples were less similar than was observed for the model standards.

The observable differences with known adenine nucleotides were shown as follows. The quantity of material needed for 1.0 optical density unit for the model compounds; AMP is 23 μg, ADP is 28 μg, and ATP is 33 μg. The toxin nucleotide required 101 μg for 1.0 OD unit. The infrared and NMR of the toxin sample when compared to AMP showed similar characteristics, but again some significant differences which could not be resolved to specific functional groups. Pyrolitic mass spectra established the presence of an adenine base, but the inorganic nature of the phosphate nucleotide was unsuitable for obtaining a parent peak.

Comparative analytical data of the adenine base, ribose sugar and phosphate content showed a ratio of 1.0 to 1.55 to 1.51, respectively.

An LD_{50} of 0.5 μg per ml was determined for the purified exotoxin using the previously reported housefly larval bioassay method.

3. A bacterial pathogen of alfalfa weevil eggs. Eggs from laboratory reared alfalfa weevils showing a high percentage of disease submitted by the Beltsville Alfalfa Weevil Investigations Laboratory, accession DD 895, were examined and found to contain the violet pigmented bacterium Chromobacterium violaceum Begonzini. Isolations made of the organism were tested against Galleria mellonella larvae and were found to be pathogenic to this insect as well. Studies on the pigment showed that it had strong antibiotic activity against Bacillus subtilis and its visible absorption spectrum was in good agreement with that cited in the literature. The strains isolated from the alfalfa weevil eggs were psychrophilic, growing well at 5° to 30°C but not at 35° or 37°C. Its failure to grow at temperatures higher than 30°C should preclude pathogenicity for warm-blooded animals that has been reported for some strains isolated and described as C. violaceum. The organism is short-lived on artificial medium and failed to transplant within 3 weeks at 15°C.

NEMATODE DISEASES OF INSECTS

1. Sterol Requirements of the DD-136 Nematode. The cooperative studies on the sterol requirements of the DD-136 were continued with the Insect Physiology Pioneering Research Laboratory. By using radioactive sterols, further insight on the mode of utilization of sterols by the nematode was obtained. The 4- ^{14}C cholestanol added to the medium was converted, mainly to 4-^{14}C- $\Delta 7$ cholestanol, with only a trace of 4-^{14}C cholesterol present at the time of analysis, indicating a rapid desaturation of the 7,8- position. The 3H-βsitosterol added to the medium was dealkylated and converted to cholesterol. The cholesterol formed was then further metabolized, saturating the 5,6 position and desaturating the 7,8 position to produce $\Delta 7$-cholestenol.

2. Improved storage of the DD-136 Nematode. Studies on storage of the nematode have produced a superior procedure for storing the nematode. When nematodes were removed from the rearing trap and held statically under refrigeration, it was found that the nematodes thus treated frequently failed to show good survival, whereas nematodes held under refrigeration

on a shaker showed consistently high survival in storage. These nematodes could then be removed from the shaker after several weeks and thereafter could be held statically under refrigeration with continued consistently high survival. The addition of sodium hydroxide to the storage fluid (Formaldehyde Solution USP 1 + 1000) also improved the storage quality of the nematode. The optimal concentration for the nematode was found to be 0.02 molar in respect to sodium hydroxide.

3. Axenic development of the DD-136 Nematode. The mass propagation of DD-136 nematode thus far has been accomplished both in the insects and on artificial medium only in the presence of its specifically associated bacterium. Either living or dead cells of the organism can satisfy the nematode, but heat killed cells failed to support its development. To obtain nematodes free of the associated bacterium, ova were surface sterilized with sodium hypochlorite. The ova were found to be quite resistant to hypochlorite and ova could be exposed to a concentration of 0.1 percent for periods as long as 55 minutes and still give some hatch. Exposure periods as short as 5 minutes gave eggs free of the bacterium. Ova exposed to 0.1 percent sodium hypochlorite for 15 minutes at 25°C consistently gave better than 50 percent hatch and were free of contamination. This was considered a practical procedure for obtaining viable bacteria-free embryos. On the artificial medium used without the addition of the associated bacterium, the eggs hatched but the larvae failed to develop further. On medium to which the associated bacterium was added, good growth and development of the nematode was obtained, thus indicating that the ova were not excessively damaged by the hypochlorite treatment.

Where surface sterilized ova of the DD-136 nematode were added aseptically to Bombyx mori tissue cultures, the nematodes developed and produced small fertile males and females, and progeny of these adults also developed to the adult stage. These cultures were free of associated bacterium. Ova added to the tissue culture medium without Bombyx mori cells hatched but the larvae failed to develop further, even though they remained active for several months in the medium.

DISEASE DIAGNOSIS

1. Virus. Only one accession received during the year contained insects that were found to be definitely virus-infected. This accession contained larvae and pupae of the corn earworm submitted to us for disease diagnosis

by R. L. Burton, Coastal Plain Experiment Station, Tifton, Ga. Midguts of all insects dissected were found heavily infected with a cytoplasmic polyhedrosis virus. Also, 70 percent of the specimens examined revealed a microsporidian infection discussed in more detail under protozoan infections. The presence of either organism alone, or in combination, could explain larval stunting and the drawn-out cycle reported in Burton's memorandum.

2. Bacteria. Thirty-one percent of the accessions received contained insects harboring bacteria suspected of producing disease. Insects from two of these accessions included bacteria belonging to the Bacillus cereus group. One of these accessions included 3 cultures of crystalliferous forms isolated from Almond moth. The cultures were submitted to us for sero-typing by W. A. McDougall of Brisbane, Australia. All 3 isolates were found to be Bacillus thuringiensis, serotype 4 (a, c) Souche-type Kenyae. The other accession containing codling moth larvae from which Bacillus cereus was isolated was received from Pesticide Chemicals Research Branch, Beltsville.

Other bacterial infections found involved either nondescript types of bacteria which did not conform to published descriptions of known species, or as yet, have not been identified. In most instances, pure cultures of these organisms were obtained, many of which were tested for pathogenicity by feeding and by injection. Bacteria of these types were isolated from cabbage looper larvae; cereal leaf beetle larvae and pupae; sesamum leaf roller larvae; alfalfa weevil eggs, larvae and adults; and tobacco hornworm larvae and pupae. Isolates of some of these forms have been added to our culture collection for future reference and study.

3. Fungi. Of the total number of accessions received during the year, 25% contained insects on which saprophytic or entomogenous fungi, or both, were found. Entomogenous forms found growing in, or on insects included: Beauveria bassiana (Bals.) Vuill. from clover root curculio and cereal leaf beetle; unidentified species of Entomophthora from pea aphids and Caribbean fruit flies; Cephalosporium ? lecanii Zimm from cereal leaf beetle; and Scopulariopsis brevicaulis (Sacc.) Bainier from Musca domestica. The latter species is not ordinarily considered infective, but under certain selective conditions it has been known to produce disease.

Fungi ordinarily considered saprophytic were found on a number of insects from accessions received and include: Penicillium spp. from cereal leaf beetle and Rhynochosciara larvae; Aspergillus nigar and A. flavus from codling moth larvae; other unidentified species of Aspergillus from the cereal leaf beetle, clover root curculio, alfalfa weevil, and sesamum leaf roller; and unidentified species of Cladosporium, Rhizopus, and Mucor on dead insects from several accessions.

Fifteen cultures of fungi isolated from the introduced pine sawfly were received for identification from H. C. Coppel, University of Wisconsin. These cultures were forwarded to Dr. C. R. Benjamin, Crops Research Division who identified 9 cultures. Specific names included: Penicillium frequentans Westl., P. terlikowskii Zaleski, P. decumbens, P. janthinellum Biourge (Z), and Aspergillus flavus Lk. ex Fr.

Isolations of many of these fungi were obtained and added to our culture collection along with permanent slides.

4. Protozoa. Accessions with insects infected with or dead of protozoan infections comprised approximately 11 per cent of those received at our laboratory. In a previously mentioned accession containing corn earworm, in which all specimens examined were found infected with a cytoplasmic virus, 70 per cent of the specimens examined also harbored a microsporidian, probably Nosema heliotidis. The principal seats of infection were found to be the silk glands and malpighian tubules.

European corn borer larvae received from R. D. Frye, North Dakota State University were found infected with Perezia pyraustae.

Two unidentified spp. of Microsporidia were found infecting the sannhemp hairy caterpillar and tobacco caterpillar in accessions forwarded to us from R. C. Patel, Institute of Agriculture, Annand, India.

5. Parasites and Undetermined Infections. Herein are included accessions of insects parasitized by other insects or arthropods with those accessions containing insects in which no disease was found.

Specimens of bagworms, Thyridopteryx ephemeraformis were found parasitized by an Ichneumonid, Hymenosynechus thyridopteryx (Riley). This accession was

received from H. M. Kulman while he was still associated with the Virginia Polytechnic Institute.

A mite, identified as Suidasia medanensi Oud. by E. W. Baker, was found heavily infesting adult Caribbean fruit flies sent to us by R. M. Baranowski, Subtropical Experiment Station, Homestead, Florida.

Accessions containing insects in which no evidence of disease could be detected or confirmed included the following: Mexican fruit fly adults received from the Plant Pest Control Division Laboratory, Monterrey, Mexico; larvae of Telea polyphemus submitted by a private individual; Bathyplectes anurus larvae in one accession and B. anurus and B. Curculionis in another received from the Insect Identification and Parasite Introduction Research Branch, Moorestown, N. J.; elm sawfly larvae submitted by R. D. Frye, North Dakota State University: alfalfa weevil larvae and adults submitted by R. L. Pienkowski, Virginia Polytechnic Institute, and from the Grain and Forage Insects Research Branch, Beltsville, Md.; cereal leaf beetle adults received from R. V. Connin, Michigan State University; field-collected adults of Hylemya lupini submitted by D. B. Leuck, Grain and Forage Insects Research Branch, Tifton, Ga.; larvae and adults of Anopheles stephansi received from Col. J. Scanlon, Walter Reed Medical Center, Washington, D.C.; omnivorous leaf roller adults submitted by A. K. Ota, Fruit and Vegetable Insects Research Branch, Beltsville, Md.; and larvae of Rhynchosciara flies submitted by E. Mattingly, Oak Ridge Laboratory, Oak Ridge, Tenn.

DISEASE CONTROL IN BEEKEEPING EQUIPMENT

1. Mortality of the Honey Bee Parasite, Nosema apis, and the Greater Wax Moth, Galleria mellonella, due to Heat Treatment. In both laboratory and field tests it was demonstrated that exposure of combs and equipment at 120°F for 24 hours effectively inactivated the spores of Nosema either in a moist or dry form. In every test, bees given unheated nosema inoculated combs came down with the disease while those given heated nosema inoculated combs were disease free at the end of the incubation period.

These tests indicate that Nosema disease can be transmitted from colony to colony by contaminated equipment and that simply heat-treating equipment at 120°F for 24 hours will inactivate the spores causing the disease.

Also all stages of the wax moth, including one day old eggs, different larval instars, prepupae, pupae, and adults exposed to 120°F for periods as short as 2 hours resulted in 100% kill. Here again this temperature treatment proved effective in ridding the combs and equipment of this insect.

INSECT TISSUE CULTURE

1. Primary Cultures. The ovaries from the silkworm, Bombyx mori, have provided one of the most reproducible systems for the primary culture of insect cells. To determine the suitability of the different types of insect cells for growth in vitro, a study of the fate of the various ovarian tissues in culture was made. It was found that silkworm ovaries maintained the integrity of their tissue in culture. Only occasionally did degenerative foci develop in the ovarioles or in the stroma of the sheath. Follicular cells and intermediate layer cells lost from damaged ovarioles appeared to have contributed most of the cells of the outgrowth around the explants. The occurrence of tracheole growth, numerous mitotic figures and meiosis in germinal tissue suggested that development continued in vitro.

The study was then expanded to compare these results with those obtained with tissue from 4 other insects Galleria mellonella, Trichoplusia ni, Spodoptera frugiperda and Estigmene acrea. Cultures were made from several stages of each insect and evaluated on the basis of the number of cells produced, the quality of the cells, and the length of life of the culture.

Comparison of the quality of the cultures with the development stage of the ovaries indicated that the intermediate cell layer and the follicular epithelium produced most of the cells in the cultures.

It was concluded that the choice of the proper stage for selection of material for ovarian cultures should be based on a compromise among these factors: large ovary size, large numbers of immature follicles and healthy intermediate layer cells, and in some species, undifferentiated ovariole sheath muscle.

2. Cell Lines. Studies have also continued on the growth of several available insect cell lines. Routine methods for maintaining adequate stocks of these lines and for producing large numbers of replicate cultures have been developed. In conjunction with members of the staff at the U.S.

Public Health Service Laboratory at Hamilton, Montana, a substrain of the Grace Antheraea cell line which did not require hemolymph in the growth medium was developed and its growth characteristics determined. Because the use of this subline is not dependent upon supplies of insect hemolymph, it has been of interest to many research workers, and cultures have been distributed to several laboratories in the United States and in other countries.

In order to further increase the volume of cells which could be grown in vitro, studies were made to develop methods for growing this adapted line in suspension cultures. By increasing the viscosity of the medium with methyl cellulose it was possible to select a cell type from the adapted cell strain which grew readily in suspension culture. This system provides the large numbers of cells, grown in a uniform environment, that are required for many studies of the biochemistry and physiology of insect cells in vitro.

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AREA NO. 20. INSECT PHYSIOLOGY AND MODE OF ACTION OF INSECTICIDES AND THEIR METABOLITES

Problem. Basic research in insect physiology is essential to the development of more efficient insecticides and new approaches to insect control. The increasing development of resistance to insecticides by insects has emphasized the need for additional information on the mode of action and metabolism of insecticides in insects and the mechanisms of the resistance to insecticides. More knowledge is also needed on the normal physiology and biochemistry of insects to permit a comparison and interpretation of the data obtained from studies on insect toxicology. Basic research in insect biochemistry and physiology, including insect nutrition, metabolism, and endocrinology will provide a better understanding of the biochemical and physiological systems which regulate insect growth, metamorphosis, reproduction and diapause, and the chemistry and action of the hormones which mediate these systems. Knowledge gained from such research is essential to the development of new methods of effective insect control which are safer and more selective in their action than the methods now being used. More basic information on the response of insects to light, sound, food, and sex attractants could contribute to better insect control. Insects are useful test animals for basic physiological studies on life processes.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program involving insect physiologists, biologists, geneticists and chemists engaged in basic studies in insect physiology and biochemistry and in the mode of action of insecticides and their metabolites. At the Pioneering Research Laboratory on Insect Physiology at Beltsville, Md., basic research is conducted on biochemistry and physiology of lipids in insects, insect hormones, and nutrition, at the Metabolism and Radiation Research Laboratory at Fargo, N. D., on metabolism of insecticides and other compounds in insects and physiological studies on insects and in the Pesticide Chemicals Research Branch at Beltsville and Bioclimatic Group at Brownsville, Texas, on insect biorhythms.

The Federal scientific effort devoted to research in this area totals 19.0 scientist man-years. Of this number 2.2 is devoted to the biochemistry and physiology of lipids in insects, 4.3 to insect hormones, 0.5 to nutrition, 2.3 to metabolism of insecticides, 2.0 to physiological processes specific to insects, 3.2 to physiology of insect growth and development and 4.5 to insect biorhythms.

Additional research in this area is provided by the following P. L. 480 projects: A7-ENT-6 India (2 professional man-years); A7-ENT-14 India (2 professional man-years); and E21-ENT-4 Poland (1 professional man-year); a research contract with the Mississippi State University, (0.3 man-year) and a grant to North Carolina State University, (0.2 man-year).

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 52.0 professional man-years is devoted to this area of research.

PROGRESS -- USDA AND COOPERATIVE PROGRAM

Insect Physiology Pioneering Research Laboratory

A. Biochemistry and Physiology of Lipids in Insects

1. Desmosterol, an Intermediate in Dealkylation of β-Sitosterol in The Tobacco Hornworm. In a study on the rate of conversion of H^3-β-sitosterol to cholesterol in the tobacco hornworm, an unknown H^3-sterol component was detected. This compound was isolated and identified as desmosterol (24-dehydrocholesterol) using IR spectroscopy, melting point determination, derivative formation, and gas-liquid chromatographic analysis by three different systems.

The H^3-desmosterol was found to be a constant metabolite of H^3-β-sitosterol in the tobacco hornworm and was observed to occur throughout larval, pupal and adult development. Desmosterol also constituted about the same percentage of the dealkylated H^3-sterols after injection of H^3-β-sitosterol, indicating that it is a metabolic product of the insect rather than of the associated microorganisms.

When hornworms were reared on a diet containing 4-C^{14}-cholesterol, no conversion of cholesterol to desmosterol was detected; thus, desmosterol is most likely an intermediate formed during the conversion of β-sitosterol to cholesterol rather than a metabolite of H^3-cholesterol formed after dealkylation. Hornworms reared on an artificial diet containing C^{14}-desmosterol as the sole source of added sterol undergo normal growth and development and analysis of the prepupae shows a nearly complete conversion of C^{14}-desmosterol to cholesterol; further substantiating desmosterol as an intermediate in the conversion of β-sitosterol to cholesterol.

The dealkylation of phytosterols to cholesterol in insects is an important biochemical mechanism which provides a means for omnivorous and particularly the phytophagous species to obtain their essential cholesterol. These studies provide us with the first information on how this transformation proceeds in insects. It is interesting that desmosterol which has a Δ^{24}-double bond, is the terminal step of dealkylation since a Δ^{24}-intermediate is involved in the primary step of alkylation in the biosynthesis of plant sterols. Work is currently underway to determine if any other intermediates in dealkylation are structurally similar to the known or proposed intermediates of the alkylation process.

2. Inhibition of the Conversion of β-Sitosterol to Cholesterol in the Tobacco Hornworm by Vertebrate Hypocholesterolemic Agents. Certain hypocholesterolemic agents, including triparanol and diazasterols have

previously been found to block the conversion of desmosterol to cholesterol in vertebrates - where this reduction is the terminal step in the de novo biosynthesis of cholesterol. The site of action of these inhibitors prompted us to examine the effect of two vertebrate hypocholesterolemic agents on the metabolism of β-sitosterol in the tobacco hornworm.

Tobacco hornworms were reared on an artificial diet with either triparanol or 22,25-diazacholesterol dihydrochloride added to a diet in which β-sitosterol was used as the sole added sterol. Both diazacholesterol and triparanol severely retarded larval growth and development and less than 3 percent of the larvae on these diets reached the prepupal stage. Analysis of the sterols of the tobacco hornworms from these diets revealed that both diazacholesterol and triparanol significantly blocked the conversion of β-sitosterol to cholesterol and brought about an accumulation of both desmosterol and unchanged β-sitosterol. Analyses of smaller and more severely inhibited larvae indicated a nearly complete interference with the dealkylation mechanism suggesting a direct relationship between inhibition of the metabolism of β-sitosterol and growth inhibition. The site of in vivo inhibition by these compounds in the hornworm was further substantiated by in vitro studies. Both triparanol and diazacholesterol inhibit the Δ^{24}-sterol reductase activity in tobacco hornworm gut tissue that converts C^{14}-desmosterol to C^{14}-cholesterol.

The striking effects of the two compounds on larval growth and metamorphosis of the hornworm may be explained in a number of ways. They could be caused by the accumulation of large quantities of desmosterol on β-sitosterol in the tissues. This increase in desmosterol and β-sitosterol is accompanied by a decrease in the cholesterol content, which could limit the availability of this precursor for the molting hormones (ecdysones) and thus potentially be another factor in growth inhibition.

Insects require a dietary source of sterol for normal growth, metamorphosis and reproduction, and plant feeding insects must derive most, if not all, of their essential cholesterol through the dealkylation of phytosterols such as β-sitosterol. This study demonstrates that in the tobacco hornworm the conversion of β-sitosterol to cholesterol can readily be blocked and that this interference severely inhibits larval growth and metamorphosis. These results suggest this to be an area worthy of intensive research both for its comparative biochemical and biomedical importance as well as for its potential in the development of chemicals that may be used to disrupt the development of plant-feeding insects.

3. Cholesterol Turnover in the House Fly. The cholesterol turnover in an insect having a short life-span was studied by following the change in specific activity of free and ester 4-^{14}C-cholesterol in male house flies for 15 days. House fly larvae were reared aseptically on a semidefined diet containing 4-^{14}C-cholesterol and the resulting male flies were segregated at eclosion and fed a diet containing unlabeled cholesterol. At 5-day intervals groups of flies were removed and the free and ester cholesterol

were separated from the lipid extracts by chromatography, and the specific activity of the sterols determined. The specific activities of the free and ester cholesterol declined linearly over the 15 days, and the specific activity of the ester cholesterol remained very close to that of the free cholesterol. A rapid equilibrium between the two pools was indicated. However, the concentration of ester in tissue decreased rapidly over the first 5 days and then remained constant; the concentration of free cholesterol did not decrease until after 5 days. Apparently dietary cholesterol is absorbed by the fly, but an overall loss of cholesterol from the tissues of the adult male house fly occurs over 15 days. Additionally, this insect seems to have a "surplus" of cholesterol ester which decreases rapidly to about two-thirds the initial quantity.

4. Separation of Sterol Acetates by Column and Thin-layer Chromatography on Silver Nitrate Impregnated Adsorbents. In studies on sterol metabolism of insects it has been necessary to separate sterols which differ in unsaturation. Since the use of silver nitrate impregnated adsorbents have been used for this type of separation involving other lipids, systems were developed in which sterol acetates could be separated by column or thin-layer chromatography. Preliminary experiments had shown that better separations were obtained with the use of sterol acetates than with free sterols. The columns were packed with silicic acid impregnated with 20% of its weight of silver nitrate. Elution was stepwise with 5% increments of benzene in hexane. This column will separate the following steryl acetates; lanosterol from 24,25-dihydrolanosterol, desmosterol from cholesterol, 7-dehydrocholesterol from cholesterol, and cholesterol from cholestanol. Thin-layer plates were prepared from a slurry of Silica Gel H and 12.5% aqueous silver nitrate solution and developed with the solvent benzene-hexane 3:5(V/V). Sterols which differed by the presence or absence of a double bond at the 5 position were separated as well as those which differed by a double bond at the 24 position; in addition, cholestanol was separable from lathosterol. Fucosterol was separable from all the other sterols tested.

B. Insect Hormones

1. Isolation and Identification of Juvabione ("Paper Factor"). Balsam firwood and products derived therefrom have been shown to prevent metamorphosis of several members of the family Pyrrhocoridae. Balsam firwood was pulverized and extracted in a large chromatographic column by perfusion with chloroform-methanol. A light mobile oil with high juvenile hormone activity for Pyrrhocoris apterus was isolated from this crude extract via column and preparative thin-layer chromatography over silica gel. The compound was assigned the trivial name of juvabione. Structural analysis of juvabione was obtained via infrared, nuclear magnetic resonance, and mass spectroscopic examination of the compound and of derivatives prepared from it. After saponification and recrystallization the acid moeity was found to be identical to todomatuic acid, a monocyclic sesquiterpene which has previously been isolated from the bisulfite treated pulpwood of Abies sachalinensis. Methylation of todomatuic acid gave a methyl ester that is

identical to juvabione.

In order to ascertain whether the juvenile hormone activity of juvabione is similar to that of other compounds, the effects of juvabione and trans, trans, 10,11-epoxyfarnesenic acid methyl ester were compared on several species of Hemiptera. The latter compound is the most active synthetic juvenile and gonadotropic hormone of known structure reported to date. At levels of 0.1 and 1.0 micrograms of the epoxide all of the Hemiptera investigated responded by giving supernumerary sixth instar nymphs and some nymphal-adult intermediates. With juvabione however, only Pyrrhocoris apterus was affected at these levels; the other insects were unaffected.

One of the most interesting aspects of this study is the discovery of a monocyclic sesquiterpene with juvenile hormone activity. Although derivatives of certain straight chain alcohols have been shown to possess very high juvenile hormone activity, most of the mimicking compounds of known structure are derivatives of the acyclic sesquiterpenoid alcohol farnesol which is the often proposed parent of such monocyclic sesquiterpenes as bisabolene, zingiberene, perezone, etc., and possibly of juvabione. It is possible that certain acyclic derivatives are active by virtue of their ability to cyclize within the insect, and if this is true it follows that certain of the natural insect juvenile hormones may also be monocyclic sesquiterpenes.

2. Hormonal Termination of Diapause in the Alfalfa Weevil (In collaboration with Alfalfa Weevil Investigations, Grain and Forage Insects Research Branch). Although diapause in the pupal stage of several orders of insects has been shown to be mediated by the endocrine glands, little information is available on the adult diapause of insects. The possibility that adult diapause in the alfalfa weevil is controlled by the corpora allata was examined by treating diapausing adult weevils with a synthetic juvenile-gonadotropic hormone. Adult weevils were treated on the venter of the abdomen with 0.1, 1, 10, 50, or 100 micrograms of trans, trans, 10,11-epoxyfarnesenic acid methyl ester in 0.5 microliters of acetone. In the groups receiving 50 to 100 micrograms of the epoxide, activity and feeding began in three days and increased from none to a very active "normal" rate within 6 to 7 days. Oviposition began on the 8th day after treatment. Although there was some activity and slight feeding by the groups that had received the lower doses (0.1 to 10 micrograms) and a few mating pairs were observed in the 10 microgram group, no oviposition occurred and all these insects returned to a diapause condition 2 to 3 weeks after treatment. Acetone treated and untreated controls remained in diapause and demonstrated no feeding, mating, or oviposition during the seven week period of observation. Although the groups treated with 50 or 100 micrograms of epoxide produced significant numbers of eggs, a definite dose response relation existed: Duration of oviposition and number of eggs produced were greater after the higher dosage. In view of the dose dependency of the ovipositional response the corpora allata of these insects may have remained inactive. These results argue in favor of the existence of an inhibition that supresses

corpora allata activity and is therefore primarily responsible for the onset and maintenance of diapause in this insect.

The ability to terminate diapause with a synthetic hormone may have several practical uses: (1) continuous rearing in the laboratory of insects that normally diapause; (2) immediate laboratory use of insects collected in a diapausing condition (the fact that parasites have emerged from treated weevils indicates that development of the parasites was governed by the physiologic condition of the host; the parasites would normally have been recovered 3 to 4 months after the onset of diapause in the host); (3) potential control of insects since termination or prevention of diapause in an insect exposes it to the consequences of a hostile environment which it normally avoids by entering diapause; thus, the insect will be in a physiologic state to feed (in the possible absence of the host plant) and to mate and to reproduce during a period of environmental stress.

3. Isolation of a New Ecdysone (20,26-Dihydroxyecdysone) From the Tobacco Hornworm. Two ecdysones, α-ecdysone and 20-hydroxyecdysone, have thus far been isolated from insects and identified, and these have approximately equal biological activity in both the Calliphora and house fly assay (House fly unit=0.005-0.006 μg). We have previously shown both of these hormones to be present in 7-day-old tobacco hornworm pupae. Further studies with this insect indicated yet another biologically active substance present in relatively small amounts. This hormone, by countercurrent distribution, column, and thin-layer chromatography was more polar in its behavior than the 20-hydroxyecdysone. The chemical, nuclear magnetic resonance, and mass spectral data indicated this more polar steroid to be 20,26-dihydroxyecdysone which differs from 20-hydroxyecdysone in the presence of an additional hydroxyl group at C-26. The 20,26-dihydroxyecdysone was 1/10 to 1/15 (House fly unit=0.05-0.075 μg) as active as α-ecdysone in the house fly assay. Since the 20,26-dihydroxyecdysone is less active than either of the two major molting hormones isolated from the tobacco hornworm, this may indicate that increased hydroxylation serves as a means for the deactivation of the ecdysones by the insect. If the 20,26-dihydroxyecdysone is indeed a metabolic product of the more active ecdysone there should be an increase in titer of this compound in the later period of pupal development, and this is currently being investigated.

Three different ecdysones have been isolated and identified from tobacco hornworm pupae at their maximum titer of molting hormone activity and both the structures and biological activity suggest that these three hormones--α-ecdysone, 20-hydroxyecdysone, and 20,26-dihydroxyecdysone--are intermediates in a biosynthetic scheme. However, each could have a specific function; the more polar hormone, perhaps, being involved in the pupal to adult molt.

Metabolism and Radiation Research Laboratory

C. Studies on the Metabolism of Insecticides and Other Compounds in Insects

1. Isolation and Characterization of Insect Esterases. It has been postulated that the action of detoxifying esterases may be involved in species selectivity in poisoning by organophosphates and in the acquired resistance of house flies to certain of these insecticides. Recent experiments have indicated a possible role of esterases in lipid metabolism in the fat body and hemolymph.

Two esterases from the cockroach were purified by the sequence of acetone powder, ammonium sulfate precipitation, heat, and ion-exchange chromatography. From their behavior on ion-exchange chromatography and starch gel electrophoresis, we believe these two esterases are fairly pure. Their phosphatase activity is less than 0.4% of the esterase activity based on their hydrolysis of naphthyl acetate and naphthyl phosphate. They are inhibited by DDVP (dimethyldichlorovinyl phosphate, a cholinesterase inhibitor), and this inhibition of naphthyl acetate hydrolytic activity is partially reversed by magnesium ions. Additional studies on these enzymes are in progress.

2. Synthesis of Chemosterilants. Several compounds related to tris-aziridinyl melamine (TEM, tretamine) were synthesized. These compounds were tested as sterilants in house flies, the boll weevil, and the tobacco budworm. As a whole, these compounds are as effective as TEM in house fly sterilization. One of these compounds, (2,6-bis-(1-aziridinyl)-pyrazine), has been most effective in sterilization of the house fly and tobacco budworm at much lower doses than required with tretamine and TEPA. These compounds are: 2,6-bis-(1-aziridinyl)-pyridine; 3,6-bis-(1-aziridinyl)-pyridazine; 2,6-bis-(1-aziridinyl)-pyrazine; and, 2,4,6-tris-(aziridinyl)-pyrimidine.

3. Analysis of Chemosterilants. The stability of TEPA in buffered solutions (phosphate; pH 6, 7, 8) is currently being studied. Methods were developed for chemical assay of total and free aziridine. Preliminary data for TEPA in 0.05 M phosphate buffer at pH 7 are as follows: At 27°C there is 75% TEPA remaining at the end of 130 hours; at 32°C there is 39% TEPA remaining at the end of 130 hours; and, at 37°C there is 25% TEPA remaining at the end of 130 hours.

TEPA in 0.1 M phosphate buffer at pH 7: At 27°C there is 15% TEPA remaining at the end of 130 hours; and, for TEPA in 0.05 M pH 8 phosphate buffer, there is 5% TEPA remaining at the end of 48 hours.

Simultaneous measurement of free aziridine released by this hydrolysis at pH 7 (0.05 M phosphate buffer) and at 27, 32, and 37° shows that free aziridine accounts for less than 0.1% of the total aziridine. It appears, then, that free aziridine is not a major product of hydrolysis of TEPA

under the conditions stated, and there is no measurable free aziridine under the pH 8 conditions mentioned.

4. Normal Biological Constituents. The normal levels and kinds of proteins and electrolytes are being studied in the hemolymph and other tissues throughout the life cycle of the cabbage looper, house fly, and eventually, the tomato hornworm. Once normal levels are determined, these insects will be treated with various chemosterilants to determine their effects on the constituents under study.

5. LDH Determination in the Developing Ovaries of House Flies. The total levels and isozymes of the enzyme LDH are being studied during oögenesis in the house fly. Once the normal patterns are determined, house flies of known stages of oögenesis will be treated with various chemosterilants to determine what effect these materials have on the enzyme levels, their isozymes, and the ovaries. Other enzymes occurring normally in the ovaries and other tissues are also under study. These include MDH, GDPH, phosphatases, and esterases.

6. Ultramicro Analytical Systems for Aziridines. An ultramicro analytical method for the analysis of total and free aziridines in insect tissues was developed. With such a system, it is possible to study the degradation of many aziridine-containing chemosterilants in the organs of individual insects. Modification of existing methods provided the basis of the ultramicro systems. Essentially all that is needed is a 10-100 µl extract of the tissue in question. Appropriate reagents of less than 100 µl are reacted with the extract, and the concentration of aziridine is read as optical density or percent transmittance on a Beckman microspectrophotometer.

7. Simultaneous Automated Analysis of Total and Free Aziridine. The modified total aziridine method of Epstein et al. 1955 (Anal. Chem. 27: 1943) and the free aziridine method of Rosenblatt et al. 1955 (Anal. Chem. 27: 1291) were adapted to an automated procedure in which either or both methods can be run independently or simultaneously. The procedures allow the kinetics of aziridine-containing chemosterilants to be studied as well as residues of such compounds in or on organisms. Preliminary studies show the methods to be almost as sensitive as the manual procedures, and they are far more reproducible.

8. Chromatographic Analysis of Insect Chemosterilants. A method for the purification of hempa by thin-layer chromatography and its ultimate analysis by gas chromatography was developed. The gas chromatographic method, with minor modifications, was tested effectively on more than 25 related compounds. This method, in conjunction with other methodologies under development, will allow studies on the degradation and metabolism of many insect chemosterilants.

9. The Effect of Chelating Agents on Trichoplusia ni. The toxicity of ethylenediamine-tetraacetic acid (tetrasodium salt) to the larvae of the

cabbage looper, Trichoplusia ni (Hübner), was studied. EDTA was used in concentrations of 0.10, 0.25, 0.35, 0.50, and 1.0% (w/w) of the Ingnoffo diet. Larvae were seeded at these concentrations at the beginning of each instar. Schmidt and Sell (1967) originally did work on this problem using concentrations of 0.05, 0.10, and 0.50% EDTA, seeding only first instar larvae. They noticed significant retardation in growth and difficulties in molting and cuticle formation between the concentrations of 0.10 and 0.50% only. This experiment covers this range more extensively and demonstrates the effects of the length of time the larvae fed on the treated media. There was a direct relationship between the percent concentration and the amount of retardation in development and between the length of time the larvae were fed the treated media and the amount of retardation. EDTA seems to have a detrimental effect on molting and cuticle formation of the cabbage looper, regardless of stage placed on the EDTA-treated media. Calcium determinations were run on the hemolymph and fecal materials of the treated and control insects to determine if the EDTA is interfering with the calcium balance of the organisms.

D. Studies of Physiological Processes Specific to Insects

1. The Monogamy Factor. Research is in progress on the isolation and characterization of the factor present in the copulatory duct of the male house fly, Musca domestica (L.) which prevents females from mating a second time. Adult male house flies were extracted with either distilled water or 95% ethanol, and a substance preventing mating was found in both extracts. Due to the high toxicity of the initial extract, it was necessary to dialyze it to remove excess salts and other low molecular weight substances before it could be bioassayed.

Attempts at purification of the active factor included the use of techniques such as heating, ammonium sulfate precipitation, dialysis, and gel filtration chromatography on Sephadex. Activity remained after heating in a bioling water bath for two minutes. Results with ammonium sulfate were variable, but approximately one-third of the activity was precipitated from a saturated ammonium sulfate solution. Crude extracts can be dialyzed and the active factor remains in the dialysis tubing; however, as the extract is purified, the active factor begins to pass through the walls of the dialysis tubing. The active factor chromatographed with the low molecular weight fraction on Sephadex. To date, active extracts that prevent mating of female house flies have been obtained from the male house fly, screw-worm fly, and black blow fly.

Extracts of tissues were prepared and injected into 3-day-old virgin female flies to study mating inhibition and mortality. A factor that inhibited mating was extracted from the reproductive systems of the male and newly mated female, male abdomens, and male copulatory duct but not from the testes or the reproductive system of virgin females. An extract of the male copulatory duct caused 50% inhibition of mating at a concentration of 4.3 tissue equivalents. An extract of male abdomens was 53% more effective

than an extract of the copulatory duct. All extracts were toxic, and toxicity was enhanced by homogenization, sonification, and oxidation.

The most effective bioassay procedure resulted when extracts were prepared from unfragmented tissues, and 1 µl of the extract containing 4 tissue equivalents was injected into the thorax of 3-day-old virgin females. Three days later, the females were tested for mating inhibition.

2. Fatty Acids in the Cockroach. As part of the background study on lipid metabolism in insects, an analysis was made of fatty acids of the neutral lipid fractions of the fat body and hemolymph in the fourth and eighth instars as well as young adults of both sexes of the American cockroach, Periplaneta americana (L.). The fatty acids were extracted with chloroform-methanol and petroleum ether, passed through a Florisil column, transesterified, and finally analyzed by gas chromatography. The major saturated fatty acids in both the fat body and hemolymph fractions were palmitic and stearic, while the major unsaturated fatty acids were palmitoleic, oleic, and linoleic, with oleic predominating.

The triglyceride fraction in both the fat body and hemolymph increased in both sexes from the fourth to the eighth instar and dropped to over half the fourth instar value in the young adult stage. A similar change was seen in the monoglyceride fraction of the fat body and hemolymph. In contrast to the triglycerides, monoglycerides, and free fatty acid fractions, the diglyceride fraction decreased in the eighth instar but again increased in the hemolymph of the young adults.

It was evident from these studies that total lipid or triglyceride analysis is not indicative of the changes occurring in all the individual glyceride and free fatty acid fractions. The changes in the diglyceride, monoglyceride, and free fatty acid fractions may reflect anabolic and catabolic changes occurring in the triglycerides. The changes with age of the percentage of saturated fatty acids in the triglycerides and free fatty acids suggested a preferential utilization by the young adult of triglycerides composed of saturated fatty acids.

3. Lipid Mobilization in Insects. Gravimetric, colorimetric, and radioisotope analyses showed that the triglycerides and free fatty acids were the major lipids released from the fat body of the American cockroach, P. americana, after incubation with hemolymph. The time-course of triglyceride release was initially rapid; within 30 to 60 minutes, however, the process slowed to a standstill. The limiting factor in release was not the fat body but the saturation of the medium. The optimum release of triglyceride occurred in medium that exceeded a concentration of 50% hemolymph, and mobilization of the triglycerides was hemolymph specific.

4. Neuromuscular Substances in Insects. A neuroactive substance was found in extracts of the crayfish, Cambarus clarkii (Girard), and the following insect species: The cockroaches, P. americana, Leucophaea

maderae (F.), and Blaberus giganteus (L.); the grasshopper, Schistocerca vaga (Scudd.), and the house fly, M. domestica. This substance in low concentrations excites the motor neuron activity in the American cockroach. It also potentiates the mechanical response of the indirectly stimulated extensor muscles of the trochanter in the same insect. There is also a high specific activity of the excitatory agent in the central nervous system of the cockroach.

Data from extraction, chromatography, and chemical analyses suggest a biogenic amine. This amine appears to be distinct from all commonly known neuropharmacologically active agents. The chemical and biological aspects between this substance and Van der Kloot's Factor S and Ostlund's catechol-4 are similar.

E. Physiological Studies on Insect Growth and Development

1. Biosynthesis of Fatty Acids in the Cabbage Looper. Essential fatty acids, those fatty acids required in the diet and usually not synthesized in vivo, are required by a number of organisms. In mammals, the essential fatty acids are linoleic, γ-linolenic, and arachidonic. In insects, linoleic or linolenic are required for normal growth, pupation, emergence, and wing development of the adult. However, in the cabbage looper, only linolenic acid is effective. In addition, the source of dietary fatty acids for insects is from plants, and the linolenic acid available would be of the alpha form and not of the gamma form, which is effective in mammals.

Using ^{14}C-labeled acetate, the biosynthesis of fatty acids was studied in cabbage looper larvae. The larvae can synthesize lauric, myristic, palmitic, stearic, palmitoleic, and oleic but cannot synthesize linoleic or linolenic. Thus, even though the larvae cannot synthesize linoleic acid in vivo, they do not require it in the diet. Structural determination of the unsaturated fatty acids by reductive ozonolysis and gas chromatography indicated that linolenic acid was the alpha isomer and thus differed from the effective acid for mammals, γ-linolenic acid.

A comparison of the fatty acid composition of the diet with that of the larvae indicated that the larvae could drastically alter the composition of the fatty acids available to them in the diet. In the diet linoleic was the major fatty acid and constituted 53.2% of the total fatty acids, while in the larvae it constituted only 10.6%. The major fatty acid in the larvae was 18:1, 40.9%, which was only 16.2% of the dietary fatty acids.

2. Oöstatic Hormone Studies. Female house flies with developing ovaries produce an oöstatic hormone that suppresses ovarian development in the secondary gonotropic cycle. Oögenesis terminates at the initiation of vitellogenesis when the oöstatic hormone is present. Hormone production ceases after oviposition, and the secondary gonotropic cycle then continues its development. The oöstatic hormone maintains the cyclicity of egg development.

This hormone has been extracted in 95% ethanol from house flies with mature eggs. The extract was taken to dryness and distilled water added to give a concentration of 3.3 tissue equivalents/µl. The extracts were bioassayed as follows: Virgin female flies were injected with 1 µl of extract when they were 24 hours old and were held for 48 hours before egg development was scored. A constant temperature of 80°F was maintained throughout the entire bioassay.

3. Relationship of Age, Ovarian Development, and the Corpus Allatum to Mating in House Flies. Populations of house flies held at 72 ± 2°F began to mate when they were 24 hours old, and 100% of the population mated by 96 hours. Ovarian development was correlated to mating as follows: Females with stage 2 ovaries never mated; stage 3 females rarely mated; most females mated between stages 4 to 10. A male confined with five virgin females of different ages (to give egg development stages of 2, 3-4, 5-7, 8-9, and 10) mated most frequently with females with stage 6 through 10 ovaries.

Females allatectomized at an age of 24 hours or less did not mate, and their ovaries did not develop. Ovariectomized females mated normally; therefore, the correlation between mating and egg development is most likely due to juvenile hormone titer. This hypothesis was substantiated further by experiments with trans, trans, 10,11-epoxyfarnesenic acid methyl ester. When this juvenile hormone mimic was topically applied in mineral oil at a concentration of 5 µg/µl to allatectomized flies, 60% of the treated flies mated, whereas only 15% of the mineral oil treated allatectomized flies mated.

4. Frozen Fly Sexing Apparatus. To expedite the sexing of frozen flies for subsequent extract preparations, a glass column with a blower at the bottom was constructed. The blower was connected to a powerstat to control the air velocity in the tube. The moving air separated the flies by weight into two main fractions. Since the males are lighter, they were blown out of the tube into a beaker, and the females remained at the bottom of the tube. Frozen flies were added to the tube in various amounts to give heights of flies in the tube of 1 to 7 cm. This was done to determine the number of flies to add to give the best sex separation. The most efficient separation of males from females was obtained when approximately 500 flies were added (a column height of 3.0 cm). This gave 85 to 96% males remaining in the top fraction and 94 to 97% females in the bottom fraction.

5. Development of Tissue Culture Medium. A chemically defined nutrient medium was developed that is capable of supporting dispersed embryo cells of the Maderia cockroach, the house fly M. domestica, and the grasshopper, S. vaga, for many weeks. This permits nutritional and endocrine studies in vitro without interference from undefined substances from the nutrient medium.

6. Monolayer Cultures of Dispersed Embryo Cells. Having developed a suitable nutrient medium, systems for preparing cell monolayer cultures from the embryos of three species of insects were developed. These monolayer cultures

are suitable for detailed studies of the effects of various biologically active substances at the cellular level. These cultures are particularly well suited for study by time-lapse cinephotomicrographic and autoradiographic techniques.

7. Embryo Organ Cultures for Rapid Screening of Biologically Active Substances. A simple and rapid system for screening biologically active compounds that employs drop cultures of cockroach embryo legs was developed. This system combines the possibility of setting up large numbers of cultures in a short period of time with the ability to read the results within 24 hours.

8. Cockroach Leg Regenerate Cultures for the Study of Endocrine Gland Interaction. This study, begun two years ago, was completed and is in manuscript at the present time. The study shows that diffusable substances in the prothoracic ganglion and the corpora allata are capable of stimulating secretion by the prothoracic gland.

9. Studies on the Effect of Hormones and Hormone Analogs on the Incorporation of Thymidine by Insect Cells In Vitro. Using monolayer cultures of cockroach embryo cells maintained ~~in a chemically~~ defined medium, it is possible to demonstrate the uptake of tritiated thymidine in vitro. The cells were grown in Rose multipurpose tissue chambers, treated with the test material, and pulsed for 24 hours with tritium-labeled thymidine. The chamber was opened, and the tissue was fixed, dipped in emulsion, exposed, and then developed. A differential count of labeled vs unlabeled nuclei gave a quantitative measure of mitotic activity.

10. Chemosterilization of the Boll Weevil. An apparatus that automatically dips insects in a given solution of a chemical at programmable time intervals was constructed. Such an apparatus should be especially useful in studies designed to determine the existence of daily or circadian rhythms of susceptibility to toxic agents or other biologically active materials such as chemosterilants and insect hormones.

Pesticide Chemicals Research Branch

F. Insect Biorhythms

1. Photoperiodism and Diapause. The wavelengths of light effective in breaking diapause in codling moth larvae are being identified at Beltsville in cooperation with instrumentation specialists of the Market Quality Research Division. Larvae in diapause were exposed to 20-nm bands of the visible spectrum from 380-750 nm (nm=nanometer=millimicron). The criterion for breaking of diapause is pupation. Isolation and identification of the pigment(s) or pigment-protein complex with absorption maxima similar to those found to be effective in breaking diapause have begun.

Disc electrophoresis is being used as a micro preparative technique for preliminary isolation of colored protein-pigment complexes. Sephadex and ammonium sulfate fractionation are being utilized for larger scale separations. Disc electrophoresis is used to determine the homogeneity of protein fractions obtained by other separation techniques. Electrophoresis patterns in the hemolymph of lepidoptera have been determined during development.

The utilization of oxygen by diapausing and prepupal lepidoptera has been measured. The data suggest that in addition to a rhythm with a 24-hour period there are rhythms with periodicities of less than 24 hours.

Since diapause is an interruption in the normal life cycle (which may be considered a rhythm with a periodicity equal to the time of the complete life cycle of the insect), chemicals that would be expected to stimulate growth and development are being injected in an attempt to break diapause in selected lepidoptera. Such compounds have included NAD, NADP, methionine, guanine, detergents, and sodium and potassium chlorides. Some enchancement of the rate of breaking of diapause was obtained with methionine, but environmental factors, such as relative humidity, also seemed to play a role.

Conversely, growth inhibitors such as puromycin and actinomycin D were utilized to induce a "pseudo-diapause". A "resting state" which simulated diapause was achieved in some European corn borer and codling moth larvae which would normally have pupated.

2. Apparatus for Simultaneous Study of Effects of Temperature, Light and Humidity on Insects. It is often important in biological work to study the effects of one physical factor, such as photoperiod, over a range of temperatures and at several relative humidities. Ordinarily, such experiments would require the use of many special, high-priced cabinets with temperature, light and humidity controls for each. An apparatus has been designed and constructed with which photoperiod studies can be carried out over a wide range of temperatures simultaneously, and at several different relative humidities. The device consists of a block of aluminum with channels at each end for circulating water and with holes drilled through the blocks in a grid pattern to hold the insects. Two constant temperature water baths, one at an elevated temperature and one at a low temperature are used to circulate warm and cold water through the upper and lower ends of the blocks, respectively. A uniform temperature gradient is set up from one end of the block to the other, with each horizontal row of holes being at a different temperature. Glycerine-water solutions flowing down a perforated sheet of filter paper placed against the backs of the blocks are used to control humidity. The front and back sides of the blocks are covered with clear plastic covers. Thus far, studies have been made with these blocks on the effects of a range of temperatures on the emergence of house flies from puparia and on breaking diapause of codling moth larvae with excellent results.

3. Photoperiod Manipulation to Damage Insects. Tobacco budworms, and Madeira cockroaches were kept under constant temperature conditions but different light-dark regimens. Their growth and development as measured by weight gain and time to pupation were determined. The insects grew and developed best under a normal 12 hour light-12 hour dark regimen and in continuous light. Short periods of light during the dark period, or a shift back and forth every four days between 12 hours light and 18 hours light in a 24-hour cycle, retarded growth. Both species of insects did poorly in constant darkness. These results suggest that the endogenous rhythm is upset by the shifting of a stimulus which would normally result in entrainment if applied at the same time each day.

4. The Circadian Rhythms of Sensitivity of Insects to Insecticides. Separate groups of comparable house flies and cockroaches were exposed in constant temperature rooms each hour on the hour around the clock to a constant dosage of a pyrethrum aerosol. Both flies and roaches were found to be most susceptible to the insecticide at 4 p.m.

Bioclimatic Cabinet Investigations

G. Insect Biorhythms

1. Photoperiod and Diapause. Diapause and non-diapause strains of the oriental fruit moth were obtained from Geneva, New York, for photoperiod studies at Brownsville, Texas. Tests in which the dark phase of the daily cycle is interrupted with light periods as a means of inhibiting diapause in this species were integrated with those already underway for Heliothis spp. Preliminary results indicate that diapause in the oriental fruit moth might be less easily influenced by light manipulation than in Heliothis spp.

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AREA NO. 21. FUNDAMENTALS OF INSECT STERILITY

Problem. Basic research on insect sterility is needed to determine if this new approach can be used to control or eradicate destructive insects, thus eliminating the hazards often associated with the application of insecticides to crops and livestock or the high cost of other insect-control measures. The sterile-male technique, involving the use of gamma radiation to produce the sterility, and the release of dominant numbers of laboratory-reared sterilized males, has been utilized successfully to eliminate the screw-worm from the Southeast. Both chemicals and radiation have been employed in the sterile release program to prevent establishment of the Mexican fruit fly in California. The use of a sterilizing chemical in combination with a bait to attract insects already in the environment has tremendous possibilities and may prove more widely useful than release of steriles, because insects need not be reared in the laboratory to outnumber native insects. If a majority of native insects can be attracted and sterilized, thus outnumbering the remaining insects in the population, the same effect may be achieved without the expense of rearing, sterilizing, and releasing sterile males. This field is not necessarily limited to the use of baits containing sterilizing chemicals which insects will eat. The insects might be attracted to a light or an odor and receive a sterilizing dose of chemical through contact. Other approaches include the production of mutations in laboratory strains of insects which would not be lethal in the laboratory but would be lethal in nature. Much additional basic work is needed on the genetics and physiology of reproduction of insect pests, and on the effects of various types of sterilants, in order to determine the possibilities inherent in these new approaches to insect control and whether or not they could be utilized to destroy the many insects of economic importance.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term research program on insect sterility and its application to control and eradication of insect pests. Basic research on the fundamentals of insect sterility is conducted at the Metabolism and Radiation Research Laboratory at Fargo, North Dakota. The research is in cooperation with Crops Research and Animal Husbandry Research Divisions and with the North Dakota Experiment Station. Research on sterility in insects produced by gamma radiation and chemosterilants directed principally toward practical application to control specific insects is also conducted at a number of field laboratories and is discussed under other areas.

The Federal scientific effort devoted to research in this area totals 6.0 professional man-years. Of this total 2.0 is devoted to basic studies on radiation sterilization, 1.8 to effects of mutagenic chemicals on reproduction and heredity, 1.2 to cellular effects from exposure to chemical

mutagens or radiation, and 1.0 to genetics of selected economically important insects.

In addition Federal support of research in this area under a grant provides for 0.5 man-years devoted to effect of radiation on insect behavior and tropisms.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 3.3 professional man-years is devoted to studies on the fundamentals of insect sterility at the State stations.

PROGRESS--USDA AND COOPERATIVE PROGRAM

A. Basic Studies on Radiation Sterilization of Insects.

Studies on the radiation sterilization of the tobacco budworm, Heliothis virescens (Fabricius), and the cabbage looper, Trichoplusia ni (Hubner), have been successfully conducted. Both sexes of the adult tobacco budworm, Heliothis virescens (F.), are sterilized by 45 krad of gamma radiation. This dose does not reduce the mating ability or competitiveness of the male although the sperm from these males are not fully competitive with untreated sperm in females which have mated to both types of males. Furthermore, females mated with sterilized males do not produce as many eggs, probably because of a significant inability of treated sperm to reach the spermathaeca of the female. These two factors (reduced competitiveness of treated sperm and reduced oviposition from females mated to sterile males) reduce the effectiveness of sterile males as measured by egg hatch in ratio tests with untreated males and females.

Tobacco budworm pupae are sterilized by 35 krad applied two days prior to emergence. Emergence and lifespan of both sexes were reduced by this dose and females produce about 10% of the control oviposition (55-70% is obtained when young adults are irradiated). The dose curve for male sterility is sigmoidal and is based on irradiation of sperm with few intermediate stages of spermatogenesis present.

When adult male cabbage loopers, Trichoplusia ni, are irradiated, a dose of 30 krads is required to sterilize the males. Adult females are sterilized by a dose of slightly under 30 krads. Radiation studies with the cabbage looper demonstrated that a male receiving a substerilizing dose of gamma radiation mated to a normal female produces progeny which are semisterile to completely sterile. The amount of inherited sterility is dependent on the amount of radiation given the original male parent. A dose of 10 krads to a male, for example, only induces approximately 15-20% sterility. However, about 50% of the progeny are semisterile and 20% completely sterile when mated to nonirradiated individuals. The F_1 males whose paternal parent received 20 krads are totally sterile and the F_1 females from the same cross average only 6-10% fertility. Sterility can also be induced in

these F_1 male progeny with doses of radiation far below those required to sterilize a normal individual. The possibility of using irradiated F_1 males from previously irradiated parents in a sterile-male eradication program is presently being investigated. It could well be that sterile males of this origin will prove to be more competitive and have a longer lifespan than normal males that require an extremely high dose of radiation to produce sterility. The cytogenetic basis of the inherited sterility in the F_1 progeny from crosses where the paternal parent received a substerilizing dose of radiation is related to the recovery of a high number of reciprocal chromosome translocations. The fact that Lepidoptera have chromosomes with diffuse centromeres or polycentric chromosomes appears to account for the reason they are both radioresistant and that such a high number of translocations are recovered.

B. Effects of Mutagenic Chemicals on Insect Reproduction and Heredity.

About 300 chemicals representing the major classes of chemical mutagens and chemosterilants were evaluated for chemosterilant activity against male boll weevils. Sterility without mortality was induced by several alkanesulfonates, aziridines and phosphoramides. These chemicals do not always kill all the spermatogonial cells. Since gonial cells are very sensitive to ionizing radiation, 3 KR of 250 KVP X-rays are used in conjunction with the chemosterilant treatment as a possible means of inducing permanent sterility in both sexes without mortality.

Adult tobacco budworms were sterilized by topical application of 20 and 40 µg (per male and female moth, respectively) of tretamine (ENT-25296) and a phosphine oxide (ENT -50716). A compound synthesized in this laboratory, 2,6-Bis-(N-aziridinyl)-pyrazine (a homolog of tretamine), sterilized males and females at 10 and 20 µg/moth, respectively. These three compounds were the most effective of the 22 tested including hempa, tepa, metepa, and apholate.

Research is underway to determine the relationship between the chemical structure of various tretamine homologs and their efficiency in inducing dominant lethal mutations in mature sperm of the house fly. The structural differences being examined are: 1) the number and arrangement of nitrogens in the triazine ring, and 2) the number and position of aziridine groups on these molecules. Results to date indicate that the relationship of the aziridinyl groups to the positions of nitrogen in the triazine ring have a definite bearing on both the quantitative and qualitative reactions of these molecules with sperm in terms of dominant lethal production. Preliminary results indicate that at least one of these homologs possesses significantly greater activity in inducing male sterility than tretamine in both the house fly and the tobacco budworm. A better understanding of the structure-activity relationship of these chemicals will hopefully lead to the development of concepts leading to the synthesis of highly specific and efficient chemosterilants.

The type of sterility induced by chemosterilants has been studied using the parthenogenetic wasp, Habrobracon. A variety of chemosterilants have been tested by either tarsal contact with a residual film, feeding the chemical in sucrose solution or by topical application to the abdomen of the wasp. In tarsal contact applications most of the alkylating agents tested produced sterility although the compounds varied considerably in effectiveness. Tretamine and metepa produced complete sterility without any evidence of sperm inactivation. Chemosterilants such as tepa and several analogs appeared to produce significant amounts of sperm inactivation.

A number of other chemosterilants were tested to determine whether sterility was due to the induction of dominant lethal mutations in the mature sperm or to sperm inactivation. Most of these chemicals were chosen because they were reported to produce sperm inactivation in some insects. Among the non-alkylating chemosterilants tested were: Hydroxytriphenyltin, 4-Imidazolin-2-one, 2-Imidazolidinone, xylohydroxyquinone, ethylene glycol, and acetyl trimethyl ammonium bromide. None of the chemicals were effective chemosterilants although at high concentrations most of them were toxic. No evidence of sperm inactivation was found with any of the above.

Several sulfonates were tested because of their effectiveness as boll weevil chemosterilants and because they represented a class of chemosterilants which had not been tested for sperm inactivating properties. Myleran (ENT-25012) was not effective by tarsal contact and could not be fed to the males, but ENT-51904, a bifunctional sulfonate, and ENT-26396, ethyl methane sulfonate, were excellent chemosterilants. Both induced dominant lethal mutations in the Habrobracon sperm, and in preliminary tests both showed evidence of producing some degree of sperm inactivation.

A nitrogen mustard (ENT-25294) sterilized the males when applied by tarsal contact and also by feeding. The sterility was due mostly to the induction of dominant lethal mutations, but positive evidence of a minor degree of sperm inactivation was found in three tests.

Studies are being conducted on the cytological aspects of sperm inactivation in Musca domestica. Chemicals which are thought to produce some degree of sperm inactivation in a variety of animals, in addition to other chemicals whose effect on male housefly germ cells has not been accessed, are being investigated. The primary areas of investigation are sperm motility, egg penetration and syngamy. Of the chemicals already tested which were found to decrease house fly egg hatchability, none appear to reduce motility of the spermatozoa. Whether these chemosterilants affect egg penetration or syngamy has not yet been fully determined.

A study of the time and causes of embryonic death in the progeny produced by chemosterilized male house flies has been initiated. Various procedures are being tested in an effort to find an efficient method of pinpointing the cessation of embryogenesis. This area of research is being pursued to permit correlations between different types of chemosterilants and their effects on embroygenesis.

C. Cellular Effects in Insects from Exposure to Chemical Mutagens or Radiation.

A study of the radiation doses required to destroy the spermatogonia and thus permanently stop sperm production was initiated for several insect species. Thus far, data has been obtained for the house fly and two species of blow flies, Phormia regina and Cochliomyia macellaria. The data on the spermatogonia will eventually be related to the radiation doses required to induce different levels of dominant lethal mutations in the mature sperm.

A continuation was made of earlier studies on the control of reproductive behavior of house flies with emphasis on the role of the male accessory fluid. These studies showed most importantly that: 1) increased oviposition as well as loss of sexual receptivity by females after mating is caused by the male accessory fluid; 2) the accessory material can produce an effect on oviposition below the leval at which it prevents female remating; 3) when matings occur in which both sexes are still relatively immature, prolonged oviposition may result in some females regaining sexual receptivity.

A study of the ultrastructure of various house fly reproductive tissues was initiated. It is hoped that such studies will provide new clues as to mechanisms involved in insect reproductive biology. Thus far, the ultrastructure of the male ejaculatory duct, which produces the accessory fluid components of the semen of house flies, has been studied in some detail. The glandular cells of the duct were found to have large numbers of ribosomes and to show other features characteristic of cells synthesizing proteinaceous material.

Histochemical studies on the house fly ejaculatory duct have been initiated with special emphasis on the secretory material produced by this structure. Preliminary tests show that at least a portion of the secretory material produced the secretory cells in the duct is of a proteinaceous nature. Future studies are planned in an effort to determine relative concentrations of certain amino acid residues that may be present in the secretory material and also to examine possible build up or depletion of these residues during the reproductive life of the male house fly.

D. Genetics of Selected Economically Important Insects.

Cytological studies of induced translocations in the house fly, Musca domestica L., showed that the five linkage groups previously reported by other workers belong to the five pairs of autosomes also previously reported. No sex-linked mutants have been found thus far. The correlation of the linkage groups to particular chromosomes as seen cytologically had not been previously attempted and was the objective in this work. The new assignment of linkage groups to particular chromosomes (made by using Perje's numbering of the house fly karyotype with X and Y representing the sex chromosomes and Roman numerals I to V representing the autosomal pairs

starting with I as the longest pair and ending with V as the shortest pair) shows the Rl marker and its associated linkage group located on chromosome I, the car marker and its linkage group on chromosome II, the bwb marker and its linkage group on chromosome III, the rb marker and its linkage group on chromosome IV, and the ocra marker and its linkage group on chromosome V.

Tests are being performed in which house flies bearing chromosome translocations are introduced into population cages. Various translocation combinations are being tried to determine which are the best combinations for decreasing fertility. Homozygous and heterozygous translocations, as well as double and triple exchanges in various combinations, are being tested to determine the feasibility of using this approach to population suppression.

E. Effect of Sterilizing Doses of Radiation on Insect Behavior and Tropisms.

The effects of sterilizing doses of radiation on the response of insects has been investigated by scientists at the University of Georgia under a grant. These studies have utilized the house fly, the secondary screw-worm fly, and the fall armyworm moth. With the house fly, temperature preference tests of irradiated and non-irradiated flies have been completed and analyzed on a total numbers basis. The analysis has shown that age is the most significant variable, with sex second and irradiation level the least significant variable. No significant difference was found in response between irradiated and non-irradiated flies. Humidity preference tests have been completed and the data is is being analyzed. In addition longevity studies and flight mill performance tests were conducted.

With the secondary screw-worm, Cochliomyia macellaria L., light test response indicated that non-irradiated flies were slightly more attracted to the light used than were irradiated flies; however, no pronounced difference was noted.

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Line Project Check List -- Reporting Year July 1, 19[illegible] June 30, 19[illegible]

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Summary of Progress	Incl. in Area ? Sub-heading
ENT b1(R)	Sugarbeet insect investigations			
ENT b1-1 (R)	Control methods and biological studies of insects and mites affecting sugarbeets	Mesa, Ariz.	Yes	11-G-1
		Twin Falls, Idaho	Yes	11-A-1 11-B-1
		Yakima, Wash.	Yes	11-A-1 11-B-1 11-C-1 11-D-1 11-F-1 11-G-1
ENT b1-3 1/ (Gr)	Reproductive behavior of the beet leafhopper and the feasibility of its suppression by chemosterilants, pheromones, on other novel agents of insect control	Riverside, Calif.	Yes	11-A-1
ENT b1-4 1/ (CA)	Control methods for sugarbeet root maggot	Fargo, N.D.	No	
CR-ENT-8 1/ (CA)	Development of sugarbeet germ plasm resistant to yellows viruses and/or aphid vectors of these viruses	Prosser, Wash.	No	
ENT b2(R)	Tobacco insect investigations			
ENT b2-1 (R)	Biological control methods and biology of insects attacking tobacco foliage	Oxford, N.C.	Yes	10-A-1,2
		Florence, S.C.	Yes	10-B-3
		Quincy, Fla.	Yes	10-A-1,3 10-D-1
		St. Croix, Virgin Islands	Yes	10-A-2
ENT b2-2 (R)	Insecticide control methods for insects attacking tobacco foliage	Oxford, N.C.	No	
		Quincy, Fla.	No	
ENT b2-3 (R)	Control methods and biology of soil insects that attack tobacco	Florence, S.C.	Yes	10-B-1,2 10-C-1
ENT b2-4 (C)	Attractants, hormones, and sterilization procedures for control of tobacco insects	Oxford, N.C.	Yes	10-E-1
		Raleigh, N.C.	No	
		Florence, S.C.	Yes	10-E-1
		Quincy, Fla.	Yes	10-E-1,2 10-F-1
		Blacksburg, Va.	No	
		St. Croix, Virgin Islands	Yes	10-A-2 10-E-1 10-F-1
ENT b2-5 (Gr)	Basic studies on the nature and significance of weather as a tool for the prediction of and behavior of field populations of insects	Clemson, S.C.	No	
ENT b2-6 1/ (CA)	Ecology and bionomics of the tobacco flea beetle, Epitrex hirtipennis (Melsheimer) in the Florida-Georgia cigar wrapper tobacco area	Quincy, Fla.	No	
ENT b3(R)	Greenhouse and ornamental plant insects			
ENT b3-1 (R)	Biology and methods of control of insects on greenhouse and ornamental plants	Farmingdale, N.Y.	Yes	12-B-1,2
		Beltsville, Md.	Yes	12-A-1 12-B-4 12-E-2,3,4
		Sumner, Wash.	Yes	12-A-2 12-B-1 12-E-1
ENT b3-4 (Gr)	Pheromones and reproduction of Thyridopteryx ephemeraeformis (Haw.)	Athens, Ga.	Yes	12-A-3
AE-ENT-2 (GR)	Influence of electromagnetic energy on green peach aphid	Lafayette, Ind.	Yes	12-E-5
ENT b4	Vegetable and berry insects			
ENT b4-1 (R)	Biology and methods of control of insects affecting beans	Charleston, S.C.	No	
		Beltsville, Md.	Yes	1-B-5 1-E-1
		Yakima, Wash.	No	
		Twin Falls, Idaho	Yes	1-B-1
		Riverside, Calif.	Yes	1-A-4

Line Project Check List -- Reporting Year July 1, 1966 to June 30, 1967

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT b4-3 (R)	Biology and methods of control of insects affecting melons and other cucurbits	Charleston, S.C.	Yes	1-B-2
ENT b4-4 (R2)	Biology and methods of control of the beet leafhopper as a pest of vegetables	Twin Falls, Idaho	Yes	1-B-2
ENT b4-5 (R)	Insects in relation to diseases of vegetables and berries	Mesa, Ariz.	No	
		Beltsville, Md.	Yes	1-H-1
				1-E-2
		Yakima, Wash.	No	
ENT b4-6	Biology, host plant relationships, and methods of control of insects that attack potato	Orono, Me.	Yes	2-A-1,2
		Presque Isle, Me.	Yes	2-B-1
				2-C-2
				2-D-1
				2-F-1
				2-G-1
				2-H-1
				2-I-1
		Yakima, Wash.	Yes	2-A-1
				2-B-1,2,3
				2-C-1,2,3
				2-D-1
				2-E-1
				2-F-1
				2-G-1
ENT b4-7 (R)	Methods of preventing deleterious residues resulting from the use of insecticides on vegetables and berries	Orono, Me.	No	
		Riverside, Calif.	No	
		Charleston, S.C.	Yes	1-B-3
		Beltsville, Md.	Yes	1-C-1,2
		Twin Falls, Idaho	No	
ENT b4-8 (R2)	Investigations on the use of natural enemies and other biological methods for the control of vegetables and berry insects	Riverside, Calif.	Yes	1-A-3,5
				1-D-2
				1-E-4
		Beltsville, Md.	Yes	1-A-6
				1-E-1,2
				1-G-5,6
				1-H-1
		Charleston, S.C.	Yes	1-B-3
				1-B-4
				1-D-2
		Yakima, Wash.	Yes	1-D-4
		Mesa, Ariz.	Yes	1-D-2
				1-E-4
ENT b4-9 (R)	Biology and methods of control of insects and mites affecting strawberries and bramble berries	Beltsville, Md.	No	
		Riverside, Calif.	No	
ENT b4-10 (R)	Superseded by b4-21			
ENT b4-12 (R)	Improvement of methods and evaluation of equipment for applying insecticides to vegetable crops	Forest Grove, Oreg.	Yes	1-F-3,4, 5,6,7
ENT b4-16	Control of insects and mites affecting vegetable and berry crops through the development of resistant plant varieties	Yakima, Wash.	Yes	1-G-12
		Beltsville, Md.	Yes	1-G-1,2,3, 4,5
		Charleston, S.C.	Yes	1-G-7,8,9
		Riverside, Calif.	Yes	1-G-2,6
ENT b4-17 (CA)	Biological control of aphids attacking potatoes	Presque Isle, Me.	Yes	2-D-1
		Orono, Me.	Yes	2-D-1
ENT b4-18 (CA)	Basic studies on the biology and behavior of Aphidius spp. and Praon spp., parasites of the pea aphid, Acyrthosiphon pisum (Harris)	Walla Walla, Wash.	Yes	1-D-4
ENT b4-19 (Gr)	Factors involved in the host association of the two-spotted spider mite, Tetranychus urticae Koch	University Park, Pa.	Yes	2-F-2
				1-G-2
				2-H-2

Line Project Check List -- Reporting Year July 1, 1966 to June 30, 1967

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT b4-20	Development of sterilization, attractants, and other specific chemical procedures for control of vegetable insects	Beltsville, Md.	Yes	1-E-1,3, 4
		Farmingdale, N.Y.	Yes	1-E-2
		Mesa, Ariz.	Yes	1-E-4
		Riverside, Calif.	Yes	1-E-4
		Charleston, S.C.	Yes	1-E-5
				1-F-2
ENT b4-21 1/	Biology and methods of control of insects affecting underground portions of vegetables	Charleston, S.C.	Yes	1-A-1,2
				1-B-4
				1-D-3
				1-E-5
				1-F-2
		Yakima, Wash.	Yes	1-C-12
CR-ENT-3 (Gr)	Basic research on insect host-plant interactions involved in plant resistance to the potato leafhopper	Ames, Iowa	Yes	2-F-3
CR-ENT-5 (Gr)	Biochemical nature of resistance of strawberries to mites	Lexington, Ky.	Yes	3-G-1
ENT 0-0-4 (AID)	Biology, ecology, and development of methods for control of insect pests of bean, peas, and other vegetable legumes in Asia	Karaj, Iran	No	
		New Delhi, India	Yes	1-A-7
ENT b5	Methods of treating plants and commodities regulated by plant quarantines			
ENT b5-1 (R)	Development of treatments for plants and commodities regulated by plant quarantines	Hoboken, N.J.	Yes	4-C-3
				4-G-2
ENT b6	Mexican fruit fly and other fruit pests in Mexico that threaten U.S. horticulture			
ENT b6-1 (R)	Biology, ecology, and methods for control of the Mexican fruit fly and citrus blackfly	Mexico City, Mex.	Yes	4-A-2
				4-B-2
				4-E-2
ENT b6-2 (R2)	Studies of lures for Mexican fruit flies	Mexico City, Mex.	Yes	4-E-2
ENT b6-4 (R)	Quarantine treatments for Mexican fruit fly infested fruits	Mexico City, Mex.	Yes	4-G-1
ENT b7	Investigations of fruit flies in Hawaii			
ENT b7-1 (R)	Ecology and biology of fruit flies and their natural enemies in Hawaii	Honolulu, Hawaii	Yes	4-A-2
		Hilo, Hawaii	Yes	4-A-2
ENT b7-2 (R)	Development of new or improved mass production methods and manipulation techniques for fruit flies and their biological control agents	Honolulu, Hawaii	Yes	4-A-2
		Hilo, Hawaii	Yes	4-A-2
ENT b7-3 (R)	Investigation of fruit fly lures and repellents in Hawaii	Honolulu, Hawaii	Yes	4-E-2
		Hilo, Hawaii	Yes	4-A-2
ENT b7-8	Development of methods for eradication and control of fruit flies in Hawaii	Honolulu, Hawaii	Yes	4-B-2
				4-C-2
				4-E-2
		Hilo, Hawaii	No	
ENT b7-9	Commodity treatments to destroy fruit flies and associated pests of quarantine importance in fresh fruits and vegetables in Hawaii	Honolulu, Hawaii	Yes	4-C-2
				4-G-1
ENT b8	Deciduous fruit and nut insect investigations			
ENT b8-1 (R2)	Studies of the codling moth and its control	Yakima, Wash.	Yes	3-A-1
				3-B-1
				3-E-1
				3-F-1
		Wenatchee, Wash.	No	
		Kearneysville, W.Va	No	
		Vincennes, Ind.	Yes	3-A-1
				3-B-1
				3-D-1

Line Project Check List -- Reporting Year July 1, 1966 to June 30, 1967

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT b8-2 (R2)	Studies of orchard mites and their control	Yakima, Wash.	Yes	3-B-2 3-D-2
		Wenatchee, Wash.	No	
		Kearneysville, W.Va.	Yes	3-B-2
		Vincennes, Ind.	Yes	3-B-2
ENT b8-3 (R)	Studies of the plum curculio and its control	Ft. Valley, Ga.	Yes	3-A-3 3-B-4 3-D-3 3-E-4
		Vincennes, Ind.	No	
ENT b8-4 (R)	Studies of borers attacking deciduous fruit trees and their control	Ft. Valley, Ga.	Yes	3-A-2,5
		Vincennes, Ind.	Yes	3-A-2 3-D-3
		Kearneysville, W.Va.	Yes	3-B-4
ENT b8-6 (R)	Studies of miscellaneous insect and mite pests of deciduous fruits and their control	Ft. Valley, Ga.	Yes	3-B-4
		Vincennes, Ind.	No	
		Yakima, Wash.	Yes	3-B-4
		Wooster, Ohio	Yes	3-A-5 3-B-4 3-D-3 3-E-4
		Wenatchee, Wash.	No	
ENT b8-7 (R)	Investigations of nut insects and mites and their control	Albany, Ga.	Yes	3-A-4 3-B-3 3-E-3 3-F-2
		Shreveport, La.	Yes	3-B-3
		Wooster, Ohio	Yes	3-A-5 3-B-4
ENT b8-8 (R)	Grape insect investigations	Wooster, Ohio	Yes	3-A-5 3-B-2 3-C-1 3-F-3
ENT b8-10	Ecology, biology, and control of the pear psylla	Yakima, Wash.	Yes	3-B-4
		Wenatchee, Wash.	No	
ENT b8-11 (Gr)	Basic studies on the influence and significance of photoperiod and light on diapause and development of the codling moth	Pullman, Wash.	Yes	3-A-1
ENT b8-12 (Gr)	Basic studies on the nature and significance of chemosterilant and attractant techniques for the eradication of the oriental fruit moth	Grand Junction, Colo.	Yes	3-E-4
ENT b8-13 (Gr)	Mass rearing and biology of the peach tree borer, Sanninoidea exitiosa (Say)	Raleigh, N.C.	Yes	3-A-2
ENT b8-14 (Gr)	Basic studies on the nature and significance of sex pheromones and gamma radiation induced sterility of the navel orangeworm Paramyelois transitella (Walker)	Berkeley, Calif.	Yes	3-E-4
ENT b8-15 (Gr)	A study on the use of chemosterilants alone and in conjunction with established attractants for the control of the apple maggot, Rhagoletis pomonella (Walsh)	Orono, Me.	Yes	3-A-5
ENT b8-16 (Gr)	Ecology of mites within pomaceous tree fruit orchards	Provo, Utah	Yes	3-A-5
ENT b8-17 (Gr)	Basic studies on the behavior of the pear psylla, Psylla pyricola (Forster)	Wenatchee, Wash.	Yes	3-A-5
ENT b8-18 (Gr)	Exploratory studies on the use of ionizing radiation and chemosterilants for the control of the pecan weevil	College Station, Tex.	Yes	3-E-3
ENT b8-19 (Gr)	Use of non-diapausing strains of tortricid species in orchard insect control	Geneva, N.Y.	Yes	3-A-5

Line Project Check List -- Reporting Year July 1, 1966 to June 30, 1967

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT b8-20 1/ (CA)	Integrated control of insect and mite pests of apple orchards	Wooster, Ohio	Yes	3-D-3
ENT b8-21 1/ (Gr)	Suppression of populations of orchard mites with pesticide resistance predators, Part 1. Selection of promising predators for rearing	University Park, Pa.	No	
ENT b9 (R)	Investigations of insect and mite vectors of deciduous tree fruit viruses			
ENT b9-1 (R)	Distribution of insects and mites in and near deciduous fruit orchards infected with virus diseases	Riverside, Calif.	Yes	3-I-1
ENT b9-2	Studies of insect vectors of phony peach virus disease and their control	Ft. Valley, Ga.	No	
ENT b9-3 (R)	Studies of mite vectors of peach mosaic virus disease, including biology, ecology, and control	Riverside, Calif.	No	
ENT b9-4 (R)	Transmission studies with possible insect and mite vectors of the latent group of stone fruit viruses	Corvallis, Oreg.	No	
ENT b9-8 (R)	Studies of possible insect and mite vectors of pear decline and their control	Riverside, Calif.	Yes	3-E-4 3-F-3 3-I-1
ENT b9-9	Transmission studies with possible insect and mite vectors of miscellaneous viruses causing diseases of deciduous fruits	Ft. Valley, Ga. Wenatchee, Wash. Corvallis, Oreg.	No No No	
ENT b9-10 1/ (CA)	Research on the biology of virus transmitting leaf hopper species	Corvallis, Oreg.	No	
ENT b10	Insects of citrus and other subtropical fruits			
ENT b10-1 (R)	Biology and methods of control of citrus mites	Orlando, Fla. Riverside, Calif.	Yes Yes	4-B-1 4-B-1 4-C-1
ENT b10-2 (R)	Biology and methods of control of scale insects, whiteflies, and mealybugs on citrus	Orlando, Fla. Riverside, Calif. Weslaco, Tex.	No Yes Yes	 4-A-1 4-E-1 4-A-1 4-B-1 4-E-1
ENT b10-3 (R)	Biology and methods of control of miscellaneous insects on citrus and other subtropical fruits	Riverside, Calif. Honolulu, Hawaii Mexico City, Mex. Orlando, Fla. Weslaco, Tex.	Yes No No Yes Yes	4-B-1 4-E-2 4-B-2
ENT b10-4 2/(R)	Superseded by ENT b10-9			
ENT b10-5 (R2)	Investigations of the biological control of citrus insects and mites	Orlando, Fla. Riverside, Calif. Weslaco, Tex.	Yes Yes Yes	4-D-1 4-D-1 4-D-1
ENT b10-6 (Gr)	Use of supplemental foods to increase populations of mite predators	Riverside, Calif.	Yes	4-A-1
ENT b10-7 (Gr)	Ecological study of the southern green stink bug, Nezara viridula (L.), with special emphasis on attractive plants as trap crops or lures	Honolulu, Hawaii	Yes	4-A-1
ENT b10-8 1/ (CA)	Biology, ecology, and control of Anastrepa suspensa (Loew)	Orlando, Fla. Homestead, Fla.	Yes Yes	4-D-2 4-D-2
ENT b10-9 1/	Insect vectors of tristeza and other diseases of citrus	Orlando, Fla.	Yes	4-I-1
ENT b11	Japanese beetle, European chafer, and related species			
ENT b11-1 (R)	Investigations of methods for controlling the Japanese beetle and eradicating isolated infestations	Moorestown, N.J.	Yes	12-B-5
ENT b11-2 (R)	Development and improvement of treatments to permit movement of nursery stock and farm products under quarantine regulations	Moorestown, N.J.	No	

Line Project Check List -- Reporting Year July 1, 1966 to June 30, 1967

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Summary of Progress	Incl. in Area & Sub-heading
ENT b11-3 (R)	Development of methods of making biological assays of insecticidal residues in soils	Moorestown, N.J.	Yes	12-C-1
ENT b11-4 (R2)	Investigations of survey methods and biological and chemical control of the European chafer	Geneva, N.Y.	Yes	12-A-5 12-B-6 12-D-2 12-F-2
ENT b11-6 2/	Superseded by ENT b11-10			
ENT b11-7 (Gr)	Basic studies of the biology of the Cuban May beetle Phyllophaga bruneri Chapine	Gainesville, Fla.	Yes	12-A-6
ENT b11-8	Development of mass production methods for the Japanese beetle, European chafer, and related species	Geneva, N.Y. Moorestown, N.J.	No. Yes	 12-A-4
ENT b11-9 1/ (CA)	Basic studies on the potential use of gamma radiation as a method for sterilization of the European chafer, Amphimallon majalis (Razoumowsky)	Geneva, N.Y.	Yes	12-E-7
ENT b11-10 1/	Attractants, sterilization techniques and biological agents for suppression or eradication of the Japanese beetle	Moorestown, N.J. Beltsville, Md.	Yes Yes	12-D-1 12-E-6 12-F-1 12-E-6
ENT c1	Boll weevil investigations			
ENT c1-1 (R)	Biological research on the boll weevil	Florence, S.C. State College, Miss. Stoneville, Miss. Tallulah, La. Waco, Tex. Brownsville, Tex. College Station, Tex. Spur, Tex. Tucson, Ariz.	Yes Yes Yes Yes Yes Yes Yes Yes Yes	9-A-1 9-A-1 9-A-1 9-A-1 9-A-1 9-A-1 9-A-1 9-A-1 9-A-1
ENT c1-2 (R)(C)	Development of more effective insecticides and formulations and more efficient application methods for control of the boll weevil	Florence, S.C. State College, Miss. Stoneville, Miss. Tallulah, La. Waco, Tex. College Station, Tex.	Yes Yes Yes Yes Yes Yes	9-B-1 9-E-1 9-B-1 9-E-1 9-B-1 9-E-1 9-B-1 9-E-1 9-B-1
ENT c1-3 (R)	Physiological and nutritional research on the boll weevil	Florence, S.C. State College, Miss. Baton Rouge, La. College Station, Tex. Tucson, Ariz.	Yes Yes Yes Yes Yes	9-A-1 9-A-1 9-A-1 9-A-1 9-A-1
ENT c1-4 (C)	Discover and develop methods other than insecticidal for controlling the boll weevil	State College, Miss. Phoenix, Ariz. Birmingham, Ala.	Yes Yes Yes	9-C-1 9-D-1 9-F-1 9-D-1 9-D-1
ENT c1-5 (C)	Discover and develop methods for eradicating the boll weevil	State College, Miss. Birmingham, Ala.	Yes Yes	9-D-1 9-D-1
ENT c1-6 (C)	Determine the number of weevils surviving the winter and the period of their emergence from hibernation quarters in a 4 county area of central Texas	College Station, Tex.	Yes	9-A-1

Line Project Check List -- Reporting Year July 1, 1966 to June 30, 1967

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT c1-7 1/ (C)	Screening investigations to find an effective boll weevil chemosterilant	Birmingham, Ala.	No	
ENT c1-8 1/ (Gr)	Number and size of spray droplets deposited on target insects as influenced by the type and density of foliage	Athens, Ga.	No	
ENT c2	Bollworm investigations			
ENT c2-1 (R)	Biological and physiological and nutritional research on the bollworm and tobacco budworm	Florence, S.C.	Yes	9-A-2
		Stoneville, Miss.	Yes	9-A-2
		Baton Rouge, La.	Yes	9-A-2
		Waco, Tex.	Yes	9-A-2
		Brownsville, Tex.	Yes	9-A-2
		Tucson, Ariz.	Yes	9-A-2
ENT c2-2 (R)	Development of more effective insecticides and formulations and more efficient application methods for control of the bollworm and tobacco budworm	Florence, S.C.	Yes	9-B-2 9-E-2
		Stoneville, Miss.	Yes	9-B-2 9-E-2
		Brownsville, Tex.	Yes	9-B-2 9-E-2
		Waco, Tex.	Yes	9-E-2
		Tallulah, La.	Yes	9-E-2
		College Station, Tex.	Yes	9-B-2
ENT c2-3 (C)	Discover and develop methods other than insecticidal for controlling the bollworm and tobacco budworm	Florence, S.C.	Yes	9-C-2 9-E-2
		College Station, Tex.	Yes	9-C-2
		Waco, Tex.	Yes	9-C-2
		Brownsville, Tex.	Yes	9-C-2 9-F-2
		Stoneville, Miss.	Yes	9-C-2
		Tallulah, La.	Yes	9-C-2
ENT c2-4 2/ (C)	Bionomics of boll weevil and bollworm populations as related to cotton insect control practices	State College, Miss.	Yes	9-C-2
ENT c2-5 (Gr)	The biology and ecology of spiders occurring in cotton fields of the San Joaquin Valley of California and their effects on populations of bollworms, lygus bugs, and other cotton pests	Davis, Calif.	Yes	9-C-2
ENT c2-6 (Gr)	Basic studies on the nature and significance of factors affecting the efficiency of Coleomegilla maculata (De Geer) as a predator of Lepidopterous eggs	Fayetteville, Ark.	Yes	9-C-2
ENT c2-7 1/ (Gr)	The influence of certain plant hormones and their balance on diapause in Heliothis zea (Boddie) and Heliothis virescens F.	Fayetteville, Ark.	Yes	9-C-2
ENT c-3	Cotton insects other than boll weevil, bollworm, and pink bollworm, and insects attacking other fiber plants			
ENT c3-1 (R)	Biological, physiological and nutritional research on miscellaneous insect and spider mites pests of cotton	Stoneville, Miss.	Yes	9-A-4
		College Station, Tex.	Yes	9-A-4
		Brownsville, Tex.	Yes	9-A-4
		Tucson, Ariz.	Yes	9-A-4
		Baton Rouge, La.	Yes	9-A-4
ENT c3-2	Development of more effective insecticides and formulations and more efficient application methods for control of miscellaneous insect and spider mite pests of cotton	Waco, Tex.	Yes	9-B-3
		Tallulah, La.	Yes	9-B-3
		Stoneville, Miss.	Yes	9-B-3
		Florence, S.C.	Yes	9-B-3
ENT c3-3	Discover and develop methods other than insecticidal for controlling miscellaneous insect and spider mite pests of cotton	Waco, Tex.	Yes	9-C-3
		College Station, Tex.	Yes	9-C-3
		Brownsville, Tex.	Yes	9-C-3
		Auburn, Ala.	Yes	9-C-3
		Tucson, Ariz.	Yes	9-C-3

Line Project Check List -- Reporting Year July 1, 1966 to June 30, 1967

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT c3-4 (Gr)	Basic studies on the nature and significance of pathogenic agents of Tetranychus spp. on cotton	Auburn, Ala.	Yes	9-C-3
ENT c4	Pink bollworm investigations			
ENT c4-1 (R)	Development of more effective insecticides and more efficient application methods for control of the pink bollworm	Brownsville, Tex. Tucson, Ariz.	No No	
ENT c4-8	Biological, physiological and nutritional research on the pink bollworm	Brownsville, Tex. Phoenix, Ariz.	Yes Yes	9-A-3 9-A-3
ENT c4-9	Discover and develop methods other than insecticidal for controlling or eradicating the pink bollworm	Waco, Tex. Brownsville, Tex. Phoenix, Ariz.	Yes Yes Yes	9-E-3 9-D-2 9-D-2
ENT c5	Corn insects			
ENT c5-1 (R)	Biology and ecology of the European corn borer	Ankeny, Iowa	Yes	7-A-1
ENT c5-2 (R)	Chemical control of the European corn borer	Ankeny, Iowa	Yes	7-B-1
ENT c5-3 (R)	Plant resistance to the European corn borer	Ankeny, Iowa Wooster, Ohio	Yes Yes	7-G-1 7-G-1
ENT c5-4 (R)	Biological control of the European corn borer	Ankeny, Iowa	Yes	7-D-1
ENT c5-5 (R)	Biology, ecology, and methods of control of the corn earworm	Tifton, Ga.	Yes	1-A-5 1-B-7 1-C-3,4,5 1-E-6 1-F-1 1-G-9 7-A-1 7-B-1 7-G-1
ENT c5-6 (R)	Biology, ecology, and methods of controlling miscellaneous insects attacking corn	State College, Miss. Tifton, Ga. Lafayette, Ind.	Yes Yes Yes	7-A-1 7-B-1 7-D-1 7-G-1 7-A-1 7-C-1 7-E-1 7-F-1 7-G-1 7-D-1
ENT c5-7 (R)	Plant resistance of corn to rice weevil attack	State College, Miss. Tifton, Ga.	Yes Yes	7-G-1 7-G-1
ENT c5-8 (R)(C)	Biology, ecology, and methods of control of soil insects attacking corn	Brookings, S. Dak.	Yes	7-A-1 7-B-1 7-F-1 7-G-1
ENT c5-9 (C)	Distribution, biology, ecology, and control of insect vectors of corn diseases	State College, Miss.	Yes	7-H-1
ENT c5-10 (Gr)	Insect transmission of viruses that cause stunting of corn	Columbia, Mo.	Yes	7-H-1
ENT c5-11	Biological control of corn rootworm and other soil insects	Brookings, S. Dak.	No	
ENT c5-12 (CA)	Chemistry of a sex attractant in the southwestern corn borer Zeadiatrea grandiosella (Dyer)	Auburn, Ala.	Yes	7-E-1
ENT c5-13 (CA)	Response of insects to radiations in the infrared and microwave regions	Berkeley, Calif.	Yes	7-E-2
ENT c5-14 (CA)	Mite transmission of corn viruses	Wooster, Ohio	No	
ENT c5-15 1/ (C)	Encapsulation as a technique in the formulation of insecticides and viruses for control of insects	Dayton, Ohio	No	
ENT c5-16 1/	Plant resistance to insects and mites associated with viral and toxicogenic diseases of corn	Wooster, Ohio	No	

Line Project Check List -- Reporting Year July 1, 1966 to June 30, 1967

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
AE-ENT-1 2/ (C)	Superseded by AE-ENT-4			
AE-ENT-3 1/ (CA)	A study of insect spines and other parts of the exoskeleton as possible detectors in the infrared region of the electromagnetic spectrum	Atlanta, Ga.	Yes	7-E-2
AE-ENT-4 1/ (C)	Investigations to determine insect response related to attraction or communication in the infrared spectral region for use in the control of insects	Ann Arbor, Mich.	Yes	7-E-2
CR-ENT-2 (Gr)	Biochemical basis for resistance of maize to attack by the European corn borer	Ames, Iowa	Yes	7-G-1
CR-ENT-4 1/ (CA)	Nature of resistance of barley and wheat to the greenbug Schizaphis graminum (Rodani)	Stillwater, Okla.	Yes	7-G-2
CR-ENT-6 1/ (CA)	Nature of resistance of corn to corn earworm	Columbia, Mo.	Yes	7-G-1
ENT-0-0-2 (AID)	Biology, ecology, and development of methods for the control of sorghum, millet, and maize insects in Africa	Zaria, No. Nigeria Serene, Uganda, Africa	Yes No	7-A-2
ENT c6	Small grain insects			
ENT c6-1 (R)	Biology, ecology, and methods of control of aphids attacking small grains	Stillwater, Okla. Brookings, S. Dak.	Yes Yes	7-G-2 7-A-2 7-G-2
ENT c6-3 (R)	Biology, ecology, and methods of control of Hessian fly and wheat jointworm attacking small grains	Tifton, Ga.. Manhattan, Kans. West Lafayette, Ind.	No No Yes	7-A-2 7-E-2 7-G-2
ENT c6-4 (R)	Biology, ecology, and methods of control of the wheat stem sawfly	Bozeman, Mont. Fargo, N. Dak.	Yes Yes	7-B-2 7-G-2 7-A-2 7-D-2 7-E-2 7-G-2
ENT c6-5 (R)	Biology, ecology, and methods of control of insects attacking sorghums	Stillwater, Okla. Tifton, Ga.	Yes Yes	7-A-2 7-B-2 7-G-2 7-G-2
ENT c6-6 (R)	Biology, ecology, and methods of control of soil insects and related pests of small grains	Brookings, S. Dak.	Yes	7-E-2
ENT c6-7 (R)	Distribution, biology, ecology, and control of insect and mite vectors of small grain diseases	Brookings, S. Dak.	Yes	7-H-1 7-H-2 8-B-1 8-H-1
ENT c6-8 (R)	Biology, ecology, and methods of control of rice field insects	Baton Rouge, La.	Yes	8-A-1 8-B-1 8-C-1 8-G-1
ENT c6-9 (C)	Biology, ecology, and methods of control of Oulema melanopa attacking small grains	East Lansing, Mich.	Yes	7-A-2 7-B-2 7-D-2 7-E-2 7-G-2
ENT c6-10 (Gr)	Behavior of cereal leaf beetle as affected by climatic factors	Lafayette, Ind.	No	
ENT c6-11 (Gr)	Microbiology and pathologies of Oulema melanopa (L.)	Columbus, Ohio	Yes	7-D-2
ENT c6-12 (Gr)	Control of damage by larvae of the rice water weevil (Lissorhopterus oryzophilus Kuschel) by increasing plant tolerance	Fayetteville, Ark.	Yes	8-G-1
ENT c6-13 1/ (CA)	Biology, life history, and propagation methods for parasites, especially ' Tetrastichus julis, of the cereal leaf beetle	Lafayette, Ind.	Yes	7-D-2

Line Project Check List -- Reporting Year July 1, 1966 to June 30, 1967

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT c7	Sugarcane insects			
ENT c7-1 (R)	Biology, ecology, and methods of control of borers attacking sugarcane	Houma, La.	Yes	11-A-2 11-B-2 11-D-2 11-E-1 11-F-1
		Canal Point, Fla.	Yes	11-A-2
ENT c7-2 (R)	Biology, ecology, and methods of control of insects other than borers attacking sugarcane	Houma, La. Canal Point, Fla.	Yes Yes	11-A-2 11-D-2
ENT c7-3 (R)	Biology, ecology, and methods of control of insect and mite vectors of sugarcane diseases	Houma, La.	Yes	11-G-2
ENT c7-4 (Gr)	Factors affecting the efficiency of Trichogramma spp. as parasites of lepidopterous pests	Baton Rouge, La.	Yes	11-D-2
ENT c8	Legume and grass insects			
ENT c8-2 (R)	Biology, ecology, and methods of control of insects attacking legumes other than alfalfa and clovers	Columbia, Mo.	Yes	6-A-1 6-B-1 6-C-1 6-D-1 6-E-1
		Tifton, Ga.	Yes	6-A-2 6-E-2
ENT c8-3 (R)	Biology, ecology, and methods of control of insects attacking grasses	Lincoln, Nebr.	Yes	5-A-4 5-B-3 5-D-3
		Tifton, Ga.	Yes	5-A-4 5-B-3 5-G-9
		Corvallis, Oreg.	No	
		University Park, Pa.	Yes	5-A-4
ENT c8-4 (R)	Insect vectors of pathogenic agents affecting legumes and grasses	University Park, Pa.	Yes	5-H-1
		Columbia, Mo.	Yes	6-D-1
ENT c8-5 (R)	Insecticide residues on forage crops	Tifton, Ga.	Yes	5-C-1,2, 3,5,6
		Beltsville, Md	Yes	5-C-1,2, 3,4 6-C-1
		Yakima, Wash.	Yes	5-C-7
ENT c8-6 (C)	Biology, ecology, and methods of control of aphids, leafhoppers, seed chalcids, and miscellaneous insects attacking alfalfa	Lincoln, Nebr.	Yes	5-A-2 5-B-2 5-G-2,5
		Tucson, Ariz.	Yes	5-A-2 5-G-2
		Manhattan, Kans.	Yes	5-G-2
		Mesa, Ariz.	Yes	5-B-2 5-G-2,4,5
		University Park, Pa.	Yes	5-G-1
ENT c8-7	Biology, ecology, and methods of control of insects attacking clover and sweetclover	Lincoln, Nebr.	Yes	5-A-3 5-G-7,8
		University Park, Pa.	Yes	5-H-1
ENT c8-8	Biology, ecology, and methods of control of the alfalfa weevil	Beltsville, Md.	Yes	5-A-2 5-B-2 5-D-2 5-F-1 5-G-1
		Lincoln, Nebr.	Yes	5-D-2
ENT c8-9 (C)	Mass production and distribution of Neodusmetia sangwani, a parasite of the Rhodesgrass scale	Weslaco, Tex.	Yes	5-D-3

Line Project Check List -- Reporting Year July 1, 1966 to June 30, 1967

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT c8-10 (Gr)	Ovipositional behavior of the alfalfa seed chalcid to chemicals occurring naturally in alfalfa	Laramie, Wyo.	Yes	5-E-2
ENT c8-11 (Gr)	Attractants and stimulants for the alfalfa weevil	Blacksburg, Va.	Yes	5-E-2
ENT c8-12 (Gr)	Resistance of alfalfa plants and varieties to tarnished plant bug and other mirid species (lygus bugs)	Manhattan, Kans.	Yes	5-G-4
ENT c8-13 1/ (Gr)	Artificial rearing of plant sucking insects (leaf bugs and aphids) on chemically defined diets under aseptic conditions	University Park, Pa.	Yes	5-A-2
ENT c8-14 (Gr)	Testing for genetic resistance to thrips in peanuts	Stillwater, Okla.	Yes	6-E-2
ENT c8-15 (Gr)	Physiology of injury caused by lygus bugs	Davis, Calif.	Yes	5-A-2
ENT c8-16 1/ (CA)	The role of insects as vectors of peanut stunt virus	Raleigh, N.C.	Yes	6-F-1
ENT c8-17 1/ (CA)	Biology and methods of control of soybean insects	State College, Miss.	Yes	6-A-1
ENT c8-18 1/ (Gr)	Bionomics of the parasite Aphidius smithi Sharma and Subba Rao, and the influence of insecticides applied for control of the alfalfa weevil on parasite populations	Lexington, Ky.	Yes	5-D-2
CR-ENT-1 (Gr)	Nature of resistance of Melilotus infesta to sweetclover weevil	Lincoln, Nebr.	Yes	5-G-8
CR-ENT-7 1/ (C)	Relationship of saponins to pest resistance in alfalfa	Research Triangle Park, N.C.	Yes	5-G-1
ENT c9	General feeder insects			
ENT c9-1 (R)	Biology, ecology, and biological methods of control of armyworms and cutworms	Baton Rouge, La.	No	
ENT c9-2 (R)	Biology, ecology, and methods of control of grasshoppers	Mesa, Ariz. Bozeman, Mont.	Yes Yes	5-A-1 5-A-1 5-B-1 5-D-1 5-G-6
ENT c9-3 (R)	Biology, ecology, and methods of control of white-fringed beetles	Gulfport, Miss.	Yes	5-A-5 5-B-4 5-D-4 5-E-1
ENT c9-4 (Gr)	The development of artificial rearing techniques for the white-fringed beetle	Auburn, Ala.	Yes	5-A-5
ENT c9-5 (Gr)	A study of viruses in grasshoppers	Bozeman, Mont.	Yes	5-D-1
ENT c9-6 (Gr)	Food habits of selected Great Plains grasshoppers inhabiting cultivated grasslands versus rangelands	Manhattan, Kans.	Yes	5-A-1
ENT c9-7 1/ (CA)	Lipid characterization of grasshopper fat bodies and eggs in relation to infection by Malamoeba locustae	Bozeman, Mont.	Yes	5-D-1
ENT c9-8 1/ (Gr)	The biology and control of the sunflower moth	College Station, Tex.	Yes	6-A-3
ENT c9-9 1/ (CA)	Evaluation of plant extracts and synthetic chemicals as attractants for the white-fringed beetle	Athens, Ga.	Yes	5-E-1
ENT c13 1/	Insect resistance in new crops (Not divided into line projects)	Ames, Iowa	Yes	6-E-2
ENT c10	Bee culture investigations			
ENT c10-1 (R)	Etiology of bee diseases and development of control methods for diseases and pests	Baton Rouge, La. Beltsville, Md. Laramie, Wyo. Tucson, Ariz. Madison, Wisc.	Yes Yes Yes Yes Yes	15-C-1,6 15-C-1,2, 3,4,7,9 15-C-1,2, 3,5,8.10 15-C-2 15-C-1,3,4

Line Project Check List -- Reporting Year July 1, 1966 to June 30, 1967

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT c10-2 (R)	Biology and breeding for improvement of the honey bee	Baton, Rouge, La.	Yes	15-A-1,2, 3,4,5,6, 9
		Logan, Utah	Yes	15-A-9
		Tucson, Ariz.	Yes	15-A-7,8
ENT c10-3 (R)	Behavior and utilization of honey bees in the pollination of agricultural and other economic crops	Tucson, Ariz.	Yes	15-D-1,2, 3,4
		Beltsville, Md.	Yes	15-D-5
ENT c10-4 (R)	Biology and utilization of insects other than honey bees in the pollination of agricultural crops	Logan, Utah	Yes	15-F-1,2,3
ENT c10-5 (R)	Effect of pesticides, insect diseases and farm practices on honey bees and other pollinating insects	Laramie, Wyo.	Yes	15-E-1
ENT c10-6	Management for improvement in productivity of honey bees	Tucson, Ariz.	Yes	15-B-7
		Madison, Wisc.	Yes	15-B-1,2,3, 4,5
		Logan, Utah	Yes	15-B-8
		Beltsville, Md.	Yes	15-B-6
ENT c10-7 (Gr)	Pathogenesis and diagnosis of Nosema disease in Apis mellifera L.	Columbus, Ohio	Yes	15-C-11
ENT c10-8 (Gr)	Glands in bees, their topography innervation, morphology, histology, and physiology	Logan, Utah	Yes	15-F-3
ENT c10-9 (Gr)	A behavioral study of the effect of hormonal secretions of the queen honey bee (Apis mellifera L.) on the industriousness of worker honey bees	Urbana, Ill.	Yes	15-C-9
ENT c10-10 1/ (CA)	Innate and acquired immunity in the honey bee	Tucson, Ariz.	Yes	15-C-12
ENT c10-11 1/ (CA)	Honey bee pollination requirements of hybrid cucumbers	East Lansing, Mich.	Yes	15-D-6
ENT h1 (R)	Mosquitoes, sand flies, and gnats investigations			
ENT h1-4 2/	Superseded by h1-29			
ENT h1-15 (R2)	Studies on the relationship of water and land management procedures to mosquito breeding in water impoundments and irrigated farming areas	Fresno, Calif.	No	
		Corvallis, Oreg.	Yes	13-F-1 14-E-1
ENT h1-17 (C)	Studies on the biology and control of salt-marsh and ricefield mosquitoes in Louisiana and other Gulf Coast areas	Lake Charles, La.	Yes	13-A-1 14-A-1
		Lafayette, La.	Yes	13-A-1 14-A-1
ENT h1-18 (C)	The effect of predators and parasites on the breeding potential of mosquitoes found in coastal-marsh areas of Louisiana	Lake Charles, La.	Yes	13-C-1 14-C-1
ENT h1-19 (Gr)	Population dynamics, sterilization, and attractants for the eye gnat, Hippelates pusio Loew	Gainesville, Fla.	Yes	13-F-2 14-E-1
ENT h1-20 (C)	Dispersal of concentrated or undiluted insecticides for increased effectiveness and economy in mosquito control	Berkeley, Calif.	Yes	13-B-1 14-B-1
ENT h1-21 (C)	Pathogens as biological control agents for mosquito larvae	Fresno, Calif.	Yes	13-C-1 14-C-1
ENT h1-22 (Gr)	Behavior and food preferences of introduced annual fishes in relation to mosquitoes	Riverside, Calif.	Yes	13-C-1 14-C-1
ENT h1-23	Biology and control of black flies, sand flies, and other gnats and their relation to disease transmission, especially on livestock and poultry	Denver, Colo.	Yes	13-G-3
		Gainesville, Fla.	No	
		Corvallis, Oreg.	Yes	14-A-1

Line Project Check List -- Reporting Year July 1, 1966 to June 30, 1967

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT h1-24	Development of effective insecticides and other materials and methods for controlling mosquitoes	Gainesville, Fla.	Yes	13-B-1 13-C-1 13-D-1 14-B-1 14-C-1 14-D-1
		Corvallis, Oreg.	Yes	13-B-1 13-C-1 13-D-1 14-B-1 14-C-1 14-D-1
		Fresno, Calif.	No	
		Lake Charles, La.	Yes	13-C-1 14-C-1
ENT h1-25	Development of repellents and other materials and improved methods to protect man and animals from mosquitoes, sand flies, and gnats	Gainesville, Fla.	Yes	13-F-1 14-E-1
		Corvallis, Oreg.	Yes	13-F-1
ENT h1-26 (Gr)	Host animals of mosquitoes and other biting Diptera in certain areas of Louisiana	Baton Rouge, La.	Yes	13-A-1 13-A-5 14-A-1 14-A-2
ENT h1-27 (Gr)	Biology, ecology, and methods of rearing several species of Culicoides found in Virginia	Blacksburg, Va.	Yes	13-A-1 14-A-1
ENT h1-28 (CA)	Studies on insecticide resistance and the sterility principle of control for insect vectors of diseases	Gainesville, Fla.	Yes	13-B-1 13-B-2 13-D-1 14-B-1 14-B-2 14-D-1
ENT h1-29 1/	Studies on the distribution, abundance, taxonomy, and biology of mosquitoes affecting agriculture	Gainesville, Fla.	Yes	13-A-1 14-A-1
		Corvallis, Oreg.	Yes	13-A-1 14-A-1
		Fresno, Calif.	No	
		Lake Charles, La.	Yes	13-A-1 14-A-1
ENT h2 (R)	Investigations on flies affecting man and livestock			
ENT h2-14 (R2)	Development of repellents and other methods to protect man from horse flies, deer flies, and stable flies	Gainesville, Fla.	No	
ENT h2-15 (R2)	Development of improved media and mass rearing and distribution techniques for screw-worm control	Mission, Tex.	Yes	13-A-8
ENT h2-16 (R2)	Development of attractants and other materials and methods for estimating and controlling natural screw-worm populations	Mission, Tex.	Yes	13-A-8 13-D-4 13-F-4
ENT h2-17 (R)	Development of physical and mechanical methods of controlling flies and other pests of livestock	Beltsville, Md.	Yes	13-B-2 13-D-2 14-B-2
ENT h2-18 (C)	Insecticidal methods for controlling the dog fly (Stomoxys calcitrans (Linnaeus) in the Gulf Coast area of Northwestern Florida	Panama City, Fla.	Yes	13-B-4 14-B-3
ENT h2-19 (Gr)	Effect of predacious mites in reducing fly production from poultry droppings	Berkeley, Calif.	Yes	13-C-2
ENT h2-20 (Gr)	Basic biology and behavior of Tabanids (horse flies)	Laramie, Wyo.	Yes	13-A-5
ENT h2-21 (Gr)	Response of the horn fly to extracts of animal tissues and to putrefaction products	Las Cruces, New Mexico	Yes	13-F-3
ENT h2-22 (Gr)	The biology and control of Tabanidae in marshlands along the Eastern shore of the Great Salt Lake, Utah	Salt Lake City, Utah	Yes	13-A-5

Line Project Check List -- Reporting Year July 1, 1966 to June 30, 1967

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT h2-23 (CA)	Insecticides, repellents, and other materials and methods for the control of horn flies, stable flies, and face flies	Kerrville, Tex.	Yes	13-A-3,4 13-B-4,5 13-C-5 13-D-5
		Corvallis, Oreg.	Yes	13-A-3,6 13-D-3
		Lincoln, Nebr.	Yes	13-C-4
		Stoneville, Miss.	Yes	13-A-4 13-B-5
		Beltsville, Md.	Yes	13-B-2
		Gainesville, Fla.	Yes	13-B-4
ENT h2-24	Development of improved larvicides and other materials and methods for the control of screw-worms and fleeceworms	Mission, Tex.	Yes	13-B-3
ENT h2-25	Development of insecticides, chemosterilants, attractants, biological agents, and other materials and methods for the control of house flies and blow flies	Gainesville, Fla.	Yes	13-B-2 13-D-2 14-B-2 14-D-2
		Corvallis, Oreg.	Yes	13-A-1,7 13-B-2 13-F-2 14-B-2 14-E-2
ENT h2-26	Studies on the biology and control of horse flies and deer flies as they relate to pests of animals and vectors of disease	Kerrville, Tex.	No	
		Lincoln, Nebr.	No	
		Stoneville, Miss.	Yes	13-A-5
		Corvallis, Oreg.	No	
		Fresno, Calif.	Yes	13-A-5
ENT h2-27 (Gr)	The influence of native parasites on the population levels of face flies and other dung-breeding Diptera	Blacksburg, Va.	Yes	13-C-4
ENT h2-28 (Gr)	Regulation of feeding in selected insects	Raleigh, N.C.	No	
ENT h2-29 1/ (Gr)	Inhibition of arthropod reproduction by organic compounds of cadmium and related materials	Stillwater, Okla.	No	
ENT h2-30 1/ (Gr)	Synthesis of fluorescent insecticides and their reaction with enzyme systems	Athens, Ga.	No	
ENT-0-0-1 (AID)(R)	Studies on the biology and control of tsetse flies in Africa	Salisbury, Rhodesia, Africa	Yes	13-D-8 14-D-4
ENT h3 (R)	Cattle grub and bot fly investigations			
ENT h3-2 (Gr)	Basic studies on the mode of action of systemic insecticides applied to sheep nose bot control	Lexington, Ky.	Yes	13-B-7
ENT h3-3	Development of new insecticides and other materials and methods for the control of grubs and bots affecting livestock	Kerrville, Tex.	Yes	13-A-9 13-B-6
		Corvallis, Oreg.	Yes	13-A-9 13-B-6
ENT h4 (R)	Lice, mites, ticks, and fleas affecting man and animals investigations			
ENT h4-7 (R2)	Development of insecticides and other methods for the control of human lice and itch mites affecting man	Gainesville, Fla.	Yes	14-B-4
ENT h4-8 (R2)	Development of insecticides and other methods for the area control of ticks, mites, and fleas with particular reference to protecting man	Gainesville, Fla.	Yes	14-B-6
ENT h4-9 (R2)	Development of repellents and other methods to protect man from mites, ticks, and fleas	Gainesville, Fla.	Yes	14-B-6
ENT h4-10 (R2)	Development of insecticides and other materials and methods for the control of ticks in sheep ked on animals	Kerrville, Tex.	Yes	13-A-10 13-B-8 13-D-7
		Corvallis, Oreg.	Yes	13-A-10
		Gainesville, Fla.	Yes	13-A-8 13-B-8

Line Project Check List -- Reporting Year July 1, 1966 to June 30, 1967

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT h4-11 (R)	Studies on the role of ticks, mites, lice, fleas, and other arthropods in the transmission of diseases of livestock and poultry	Stoneville, Miss. Beltsville, Md.	Yes Yes	13-G-1 13-G-1
ENT h4-12	Studies on the role of ticks and other arthropods in the transmission of equine piroplasmosis and on the development of insecticides and other means of controlling or eradicating vectors of the disease	Kerrville, Tex. Beltsville, Md.	No Yes	 13-G-2
ENT h4-13 (Gr)	Biological studies on the mite Neoschongastia americana (Hirst) on turkeys in Georgia	Athens, Ga.	Yes	13-A-11
ENT h4-14 (Gr)	Effects of chemosterilants on the northern fowl mite	State College, Miss.	Yes	13-D-9
ENT h4-15	Development of improved insecticides and other materials and methods for the control of lice affecting livestock	Stoneville, Miss. Kerrville, Tex. Lincoln, Nebr.	No Yes No	 13-D-6
ENT h4-16	Development of improved materials and methods for the control of external parasites of poultry	Corvallis, Oreg. Kerrville, Tex.	No Yes	 13-B-9
ENT h7	Toxicity and residue studies on insecticides and repellents in relation to the control of insects affecting livestock			
ENT h7-2 (R2)	Extent of storage of insecticides in animal tissues and amount secreted in milk of dairy cattle when used for insect control	Kerrville, Tex.	Yes	13-E-1
ENT h7-4 (R2)	Develop quantitative bioassay methods for analysis of insecticidal chemical residues	Kerrville, Tex.	No	
ENT h7-5	Investigations relating to the acute and chronic toxicity of insecticides, repellents, and other materials to livestock	Kerrville, Tex.	Yes	13-E-1
ENT h10	Household insect investigations			
ENT h10-1 (R2)	Development of measures for the control of insects in homes	Gainesville, Fla. Kerrville, Tex. Corvallis, Oreg.	Yes Yes Yes	14-B-4 14-B-8 14-E-5 14-D-3 14-A-3
ENT j1 (R)	Identification and classification of insects			
ENT j1-1 (R2)	Identification and classification of hemipterous insects	Washington, D.C.	Yes	17-A-5 17-A-8 17-C-2 17-C-6
ENT j1-2 2/ (R2)(C)	Identification and classification of beetles	Washington, D.C. College Station, Tex.	Yes Yes	17-A-1 17-C-5 17-A-1
ENT j1-3 (R2)	Identification and classification of moths and butterflies	Washington, D.C.	Yes	17-A-4 17-C-1
ENT j1-4 (R2)	Identification and classification of grasshoppers and allied insects	Washington, D.C.	Yes	17-C-4
ENT j1-5 (R2)	Identification and classification of two-winged flies	Washington, D.C.	Yes	17-A-6 17-C-7
ENT j1-6 (R2)	Identification and classification of thrips	Washington, D.C.	Yes	17-A-2
ENT j1-7 (R2)	Identification and classification of hymenopterous insects	Washington, D.C.	Yes	17-A-3 17-A-7 17-C-3 17-C-8
ENT j1-8 (R2)	Identification and classification of mites, chiggers, and ticks	Washington, D.C.	Yes	17-C-9
ENT j1-11 (Gr)	Basic studies on the taxonomy, morphology, and ecology of cutworm larvae	Ithaca, N.Y.	Yes	17-A-4
ENT j1-12 (Gr)	Basic studies of the nature and taxonomic significance of morphological characters of females of leafhoppers	Raleigh, N.C.	Yes	17-A-4 17-C-2

Line Project Check List -- Reporting Year July 1, 1966 to June 30, 1967

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT j1-13 (Gr)	Basic studies on the morphology of insect receptors stimulated by attractants	New Brunswick, N.J.	No	
ENT j1-14 1/ (C)	Index to Fascicle VI, General Catalog of the Homoptera	Raleigh, N.C.	Yes	17-C-2
ENT j1-15 1/ (Gr)	Basic research on the taxonomic and host relations of parasitic insects, subfamily Porizontinae, family Ichneumonidae	Ann Arbor, Mich.	No	
ENT j1-16 1/ (Gr)	Basic studies on the taxonomy and biology of the immature stages of the genus Acrobasis	Raleigh, N.C.	No	
ENT j2	Utilization of insect enemies in the control of insect pests and weeds			
ENT j2-6 2/	Biological control of weeds	Albany, Calif.	No	
ENT j2-7 2/ (R)	Search for and importation of foreign parasites and predators of insect pests	Paris, France	No	
ENT j2-8 (R)	Search for and importation of foreign insect enemies of weeds	Rome, Italy	Yes	18-B-3 18-C-11
ENT j2-9	Receipt and distribution of foreign natural enemies of insect pests and weeds	Buenos Aires, Argentina	Yes	18-C-9
		Moorestown, N.J.	Yes	18-A-1 18-D-1,2, 5
		Albany, Calif.	No	
ENT j2-9 2/ (C)	Investigations on the micro-organisms which attack Oulema species in Europe and the propagation and release of European insect parasites of Oulema species in Indiana	Lafayette, Ind.	No	
ENT j2-10 (C)	A study of the insects that feed on rangeland weeds of foreign origin in the State of Idaho	Moscow, Idaho	Yes	18-C-12
ENT j2-11 (Gr)	The biologies and host relationships of tachinid parasites of insects in the State of Washington	Pullman, Wash.	Yes	18-C-7
ENT j2-12 (C)	A world review of parasites, predators, and pathogens introduced into new habitats against insects and weeds	Riverside, Calif.	Yes	18-A-6
ENT j2-13 (Gr)	Selection and developments of superior strains of predators and parasites	Columbia, Mo.	Yes	18-A-14
ENT j2-14 (Gr)	Studies on the significance of the life history and ecology of Lebia analis Dej., an important predaceous ground beetle	Fayetteville, Ark.	Yes	18-C-5
ENT j2-15 (Gr)	Insects associated with aquatic weed pests of foreign origin in Louisiana	Baton Rouge, La.	Yes	18-C-9,10
ENT j2-16 (Gr)	The attraction and concentration of insect predators by nontoxic chemical stimuli	St. Paul, Minn.	Yes	18-C-15
ENT j2-17 (Gr)	Studies on the host specificity of a hymenopterous parasite Lygus (Hemiptera)	Storrs, Conn.	Yes	18-C-1
ENT j2-18 (C)	An annotated bibliography and host catalog of North American Tachinidae	San Francisco, Calif.	Yes	18-C-7
ENT j2-19 (Gr)	Basic studies on the nature and significance of the effectiveness of the parasitic wasp Tetrastichus incertus in controlling infestations of the alfalfa weevil	Ithaca, N.Y.	No	
ENT j2-20 1/	Biological control of weeds	Albany, Calif.	Yes	18-B-1,2
ENT j2-21 1/	Search for and importation of foreign parasites and predators of insect pests	Paris, France	Yes	18-D-3
ENT j2-22 1/	Screening of candidate insecticides to determine their effect on the parasites and predators of insects and spider mites	Columbia, Mo.	No	
		Riverside, Calif.	No	
ENT j2-23 1/	Identification of the factors determining the effectiveness of insect parasites and predators	Columbia, Mo.	Yes	18-D-4
ENT j2-24 1/	Principle of manipulating nutritive resources to increase or maintain effective populations of natural enemies of insects	Columbia, Mo.	No	
		Riverside, Calif.	No	

Line Project Check List -- Reporting Year July 1, 1966 to June 30, 1967

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. In Summary of Progress	Line Proj. Incl. In Area & Sub-heading
ENT j2-25 1/	Principles of designing integrated control system	Columbia, Mo.	No	
		Riverside, Calif.	No	
ENT j2-26 1/	The effect of laboratory environments and inadvertent genetic selection on the behavior and effectiveness of parasites and predators	Columbia, Mo.	No	
		Riverside, Calif.	No	
ENT m1 (R)	Chemical investigations of products of natural origin for insect control			
ENT m1-14 (R)	Investigations of plants as sources of insecticides, synergists, insect repellents or attractants, or insect antimetabolites	Beltsville, Md.	Yes	16-A-2
		State College, Miss.	Yes	16-A-2
ENT m1-15 (R)(C)	Investigation of substances naturally occurring in insects that might be used to upset their development or reproduction or otherwise affect their vital processes	Beltsville, Md.	Yes	16-A-1
		Yakima, Wash.	Yes	16-A-1
		State College, Miss.	Yes	16-A-1
		Kerrville, Tex.	Yes	16-A-1
ENT m1-16 (Gr)	Isolation, purification, and characterization of the sex attractant for the tobacco budworm, Heliothis virescens	Ann Arbor, Mich.	Yes	16-A-1
ENT m1-17 2/ (Gr)	Investigation of the tobacco hornworm sex attractant	Madison, Wis.	Yes	16-A-1
ENT m1-18 1/ (Gr)	Preparation of long-chain unsaturated alcohols and their derivatives for test as insect sex attractants	Washington, D.C.	No	
ENT m1-19 1/ (CA)	Isolation, purification, and investigation of the molecular structure of the sex attractant of the tobacco hornworm (Manduca sexta).	Madison, Wis.	No	
ENT m2 (R)	Chemical investigations to develop synthetic organic materials for insect control			
ENT m2-13 (R2)	Chemical investigations of radioactively labeled insect control agents	Beltsville, Md.	Yes	16-B-1 16-E-2
		State College, Miss.	Yes	16-B-1
ENT m2-15 (R)	Preparation of synthetic organic compounds for testing as insect control or eradication agents through effects other than death	Beltsville, Md.	Yes	16-B-1
		Gainesville, Fla.	Yes	16-B-2
ENT m2-16	Preparation of compounds for testing as insect chemosterilants	Beltsville, Md.	Yes	16-B-1
ENT m2-17	Development of basic chemical information on insect chemosterilants	Beltsville, Md.	Yes	16-B-1
		State College, Miss.	Yes	16-B-1
		Gainesville, Fla.	Yes	16-B-1
ENT m2-18 (C)	Synthesis of organic compounds for use in investigations on insect attractants and chemosterilants	Kansas City, Mo.	Yes	16-A-1 16-B-1 16-B-2
ENT m2-19	Preparation of synthetic organic compounds for evaluation as insecticides and synergists	Beltsville, Md.	Yes	16-B-2
		Gainesville, Fla.	Yes	16-B-2
ENT m2-20 (C)	Determination of toxicological properties of materials under investigation as chemosterilants, attractants, or other new types of insect control agents	Falls Church, Va.	Yes	16-B-1
ENT m2-21	Development of formulations of materials for insect control	Beltsville, Md.	Yes	16-B-3
		Gainesville, Fla.	Yes	16-B-3
		College Station, Tex.	Yes	16-B-3
		Kerrville, Tex.	Yes	16-B-3
ENT m2-23 1/ (Gr)	Synthesis of possible chemosterilants	Philadelphia, Pa.	No	
ENT m3 (R)	Analysis of pesticides, pesticide residues, and accessory materials			
ENT m3-5 (R2)	Analysis of insect control chemicals, their formulations, and accessory materials	Beltsville, Md.	Yes	16-C

Line Project Check List -- Reporting Year July 1, 1966 to June 30, 1967

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT m3-6	Determination of residues of insect control chemicals in plant and animal products and in soils	Beltsville, Md.	Yes	1-C-1,2 5-C-4 6-C-1 13-E-1
		Tifton, Ga.	Yes	1-C-3,4,5 5-C-1,2,3, 5,6 7-C-1 13-E-1
		Kerrville, Tex.	Yes	13-E-1
		Yakima, Wash.	Yes	2-C-1,2 4-C-1 5-C-7 11-C-1 13-E-1
ENT m4	Chemical investigations on fumigants and aerosols for control of insect pests			
ENT m4-1 2/	Superseded by ENT m4-11			
ENT m4-11 1/	Development of aerosols and formulations of aerosols to control insects	Beltsville, Md.	Yes	16-D
ENT m9 (R)	Laboratory tests to determine the effectiveness of insect control materials			
ENT m9-1 (R2)	Comparison of the toxic, attractant, arrestant, and repellent action of chemical materials to test insects	Beltsville, Md. Brownsville, Tex.	Yes Yes	16-E-1 16-E-1
ENT m9-3 (R2)	Comparison of insecticidal materials in gas-propelled aerosols and space sprays	Beltsville, Md.	Yes	16-E-3
ENT m9-4 (R2)	Biological evaluation and biochemical studies of materials for insect control through effects other than death	Beltsville, Md.	Yes	16-E-2 20-F-1,2, 3,4
		Brownsville, Tex.	Yes	16-E-1
ENT m10	Methods for disinsectization of aircraft (not divided into line projects)	Beltsville, Md. Brownsville, Tex.	Yes Yes	16-F 16-F
ENT m11 (R)	Development of methods of analysis for pesticides and pesticide residues			
ENT m11-2 (R2)	Development of methods of analysis for insect-control chemicals	Beltsville, Md. Tifton, Ga. Kerrville, Tex. Yakima, Wash. College Station, Tex.	Yes Yes Yes Yes Yes	16-C 16-C 16-C 16-C 16-C
ENT p1	Insect Pathology Laboratory	Beltsville, Md.	Yes	19-A-1,2, 3,4,5,6,7 19-B-1,2,3 19-C-1,2,3 19-D-1,2,3 4,5 19-E-1 19-F-1,2
ENT p2	Insect Physiology Laboratory	Beltsville, Md.	Yes	20-A-1,2, 3,4 20-B-1,2,3
ENT q1	Insect metabolism and physiology			
ENT q1-1	Studies on the metabolism of insecticides and other compounds in insects	Fargo, N. Dak.	Yes	20-C-1,2, 3,4,5,6, 7,8,9
ENT q1-2	Studies on the physiological processes specific to insects	Fargo, N. Dak.	Yes	20-D-1,2, 3,4
ENT q1-3	Physiological studies on insect growth and development	Fargo, N. Dak.	Yes	20-E-1,2, 3,4,5,6, 7,8,9,10
ENT q2	Radiation biology and insect genetics			
ENT q2-1	Basic studies on radiation sterilization of insects	Fargo, N. Dak.	Yes	21-A
ENT q2-2	Effects of mutagenetic chemicals on insect reproduction and heredity	Fargo, N. Dak.	Yes	21-B

Line Project Check List -- Reporting Year July 1, 1966 to June 30, 1967

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT q2-3	Cellular effects in insects resulting from exposure to chemical mutagens or radiation	Fargo, N. Dak.	Yes	21-C
ENT q2-4	Genetics of selected economically important insects	Fargo, N. Dak.	Yes	21-D
ENT q2-5 (Gr)	Investigations of changes in insect behavior and tropisms resulting from sterilizing doses of radiation	Athens, Ga.	Yes	21-E
ENT r1	Investigations of insect diseases			
ENT r1-1 (C)	Evaluation of vertebrate toxicity and pathogenicity of insect virus diseases	Chicago, Ill.	No	
ENT r1-2 (C)	Development of methods for the continuous production of virus-susceptible insects and virus disease organisms	College Park, Md. New Brunswick, N.J. Columbus, Ohio	No No No	
ENT r1-3 (C)	Feasibility of growing nuclear polyhedrosis virus from Heliothis zea in bacterial cells or protoplasts	Kansas City, Mo.	No	
ENT r1-4	Methods of mass-producing nuclear polyhedrosis virus-infected populations of the beet armyworm and yellow-striped armyworm, and selection of virulent mutants of neclear polyhedrosis virus disease of corn earworm	Buena Park, Calif.	No	
ENT r1-5	Determination of the virulence, pathogenicity, and interrelations between two species of viruses coexisting in the cabbage looper	Geneva, N.Y.	No	
	P. L. 480 Projects			
A6-ENT-4	Biological control of citrus, tobacco, and vegetable aphids	Taiwan	Yes	18-A-2
A7-ENT-1 2/	Investigations of parasites, predators, and pathogens of sugarcane borers in India	India	Yes	18-C-3
A7-ENT-2 2/	Survey of beneficial parasites and predators of agricultural and horticultural crops in the Indian Union	India	No	
A7-ENT-6	Nutritional studies on the silkworm Bombyx mori L. - its requirements for vitamins and amino acids and its nutrition in relation to the mineral nutrition of its host plant, mulberry (Morus indica) and studies on the host specificity of the silkworm Bombyx mori L.	India	No	
A7-ENT-7	Survey for natural enemies of witchweed, and of India waterhyacinth and other weeds affecting waterways in India	India	Yes	18-B-5
A7-ENT-8	Developing methods for large-scale rearing of parasites under laboratory conditions	India	Yes	18-C-16
A7-ENT-9 2/	Investigations of parasites, predators, and pathogens of the European corn borer and Heliothis spp. in India	India	Yes	18-A-3
A7-ENT-10 2/	Acarine disease problem of honey bees	India	Yes	15-C-13
A7-ENT-14	Studies on the free amino acids of insect haemolymph and the accumulation of citric acids in insect tissue	India	No	
A7-ENT-17	Control of the coconut rhinoceros beetle Oryctes rhinoceros L.	India	Yes	18-C-6
A7-ENT-19	Biology, ecology, and utilization of insects other than honey bees in the pollination of agricultural crops	India	Yes	15-F-4
A7-ENT-20	Studies of microbiology and pathology of insect pests of crop plants	India	No	
A7-ENT-22	Studies of Indian Jassidae with particular reference to Circulifer and related genera and their importance as vectors of plant virus diseases	India	Yes	11-G-1

Line Project Check List -- Reporting Year July 1, 1966 to June 30, 1967

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
A7-ENT-24	Systematic and biological studies of Indian thrips	India	Yes	17-A-2
A7-ENT-25	Research on insect pests of maize with special reference to stalk borers	India	Yes	7-G-1
A7-ENT-26	Biology of gall midges affecting mangoes with special reference to the extent of damage	India	Yes	4-A-1
A7-ENT-28	Taxonomic studies of several families of Mallophaga (chewing lice)	India	No	
A7-ENT-29	A study of the taxonomy of adult and larval Bruchidae	India	Yes	17-A-1
A7-ENT-31	Investigations of insect pests of sorghum and millets	India	No	
A7-ENT-33	Hereditary variations in the ability of Myzus persicae to transmit potato leafroll and virus "Y"	India	No	
A7-ENT-35	Biology of gall midges affecting citrus plants with special reference to extent of damage	India	Yes	4-A-1
A7-ENT-36 1/	Taxonomic survey of encyrtid parasites (Encyrtidae: Hymenoptera) in India	India	Yes	17-A-7
A7-ENT-37	Taxonomic survey of the hymenopterous parasites belonging to the family Ichneumonidae in India	India	Yes	17-A-7
A7-ENT-40	A study of factors affecting the dissemination of Coccinella septempunctata	India	Yes	18-C-4
A7-ENT-42	Survey for natural enemies of aphids in India	India	Yes	18-D-1
A7-ENT-44	Physiological factors governing susceptibility or resistance of crop plants to leafhoppers	India	Yes	1-G-10
A7-ENT-45 1/	Scheme to study factors affecting winter (off season) survival of pink bollworm (Platyedra gossypiella S.) of cotton in Gujarat State	India	No	
A7-ENT-47	Biology of gall midges affecting fig fruits with special reference to the extent of damage and its relationship to the spread of disease organisms	India	Yes	4-A-1
A7-ENT-51	Studies on the systematics of the aphid genus Microsiphum (Homoptera-Aphidae)	India	No	
A7-ENT-54 1/	Study on sex attractants in lepidopterous borer pests of sugarcane	India	No	
A7-ENT-57	Biochemical studies of certain insect vectors of human diseases with special reference to the development of resistance to insecticides	India	No	
A7-ENT-58 1/	Studies on the biology and taxonomy of the Agromyzidae (Diptera)	India	Yes	17-A-6
A7-ENT-59 1/	Taxonomic studies of the oriental species of Brachymaria (Hymenoptera: Chalcididae)	India	No	
A7-ENT-60	Studies on certain plant extracts and isolates having pesticidal properties	India	No	
A7-ENT-62 1/	Studies on the ecology and bionomics of parasites of sugarcane borers in India	India	Yes	18-C-3
A7-ENT-69 1/	Biology and breeding techniques for parasites and predators of Ostrinia spp. and Heliothis spp. (corn borers and earworms).	India	No	
A7-ENT-70 1/	Studies on the taxonomy, bionomics, ecology, and genetics of different species and races of Trichogramma (a parasite of moth eggs)	India	No	
A10-ENT-5 2/	Host plant-vector and host plant-virus relationships of the rough dwarf virus of corn and methods for control of the disease	Israel	Yes	7-H-1
A10-ENT-6	Acoustic responses of certain locusts (Schistocerca gregaria), Moroccan locust (Dociostaurus maroccanus) and Acrotylus insurbricus) (Orthoptera: Acrididae)	Israel	Yes	5-A-1

Line Project Check List -- Reporting Year July 1, 1966 to June 30, 1967

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
A10-ENT-10	Biology of natural enemies of citrus scale insects, in order to develop methods of their mass production for biological control	Israel	Yes	18-A-4
A10-ENT-11 1/	Action of repellents against arthropods affecting man	Israel	No	
A10-ENT-12	Laboratory study of tick repellents and acaricides	Israel	Yes	13-B-8
A10-ENT-13	Factors influencing variations in insecticide resistance	Israel	Yes	3-B-2
A10-ENT-15	A study of the ecology, biology, and control of the citrus bud mite (Aceria sheldoni) Eriophyidae	Israel	No	
A10-ENT-21 1/	The phenomenon of periodic acquisition of tomato yellow leaf curl virus by its vector, the tobacco whitefly, Bemisia tabaci Gennadius	Israel	No	
A10-ENT-24 1/	Biological and ecological studies on Myiopardalis pardalina Bigot and trials with attractants	Israel	No	
A13-ENT-3	Investigations on the biology of dung beetles in Korea and their role in the prevention of fly breeding in dung	Korea	Yes	14-C-3
A17-ENT-7 2/	Investigations on the natural enemies of corn borers	Pakistan	No	
A17-ENT-8 2/	Studies on the natural enemies of insect pests of rice	Pakistan	Yes	18-C-8
A17-ENT-10	Studies on oriental leafhoppers (Typhlocybinae)	Pakistan	Yes	17-A-8
A17-ENT-13	Insects, other plant-feeding organisms or plant diseases which attack Eurasian water-milfoil	Pakistan	Yes	18-B-4
A17-ENT-14	Biologies and host plant ranges of insects that attack noxious weeds common to Pakistan and the United States	Pakistan	Yes	18-C-13
A17-ENT-15	Basic studies of parasites of the green peach aphid in northern West Pakistan	Pakistan	Yes	18-A-5
A17-ENT-16	Relations between the parasite-predator complex and the host plants of scale insects in Pakistan	Pakistan	Yes	18-C-2
A17-ENT-19 1/	Parasites, predators, and pathogens of stink bugs (Pentatomidae) that attack weedy and cultivated graminaceous plants	Pakistan	No	
A17-ENT-20 1/	Natural enemies of forage and grain legume aphids in Pakistan	Pakistan	Yes	18-A-2
E11-ENT-1 2/	Control of the olive fly, (Dacus oleae (Gamelin) with radiation or chemical sterilization procedures	Greece	Yes	18-A-2
E15-ENT-1 2/	A study of acarine disease of honey bees	Italy	Yes	15-C-13
E21-ENT-4 2/	The causes and the role of diapause of insect pests	Poland	No	
E21-ENT-8	Mite fauna of orchards with special reference to the relation between phytophagous and predaceous species	Poland	Yes	3-D-2
E21-ENT-9 2/	Insect vectors of virus diseases of various forage legumes	Poland	Yes	5-H-3
E21-ENT-10	Studies on distance of mating flights of honey bee queens and drones	Poland	Yes	15-A-11
E21-ENT-11	Role of parasitic Hymenoptera in reduction of population size of two species of the genus Lygus	Poland	Yes	18-C-1
E21-ENT-12	The role of nematodes as factor reducing populations of insect pests	Poland	No	

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Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
E21-ENT-14	Studies regarding the bionomics, economic importance and natural control factors affecting Oulema species (cereal leaf beetle) in Poland	Poland	Yes	7-D-2
E21-ENT-15	Biology and utilization of diploid drone honey bees	Poland	Yes	15-A-10
E21-ENT-16	The population trends in predaceous arthropods in apple orchards sprayed with different pesticides and the influence of these trends on the population density of phytophagous mites and some other pests	Poland	Yes	3-A-5
E21-ENT-17	Studies on interactions of various pathogens in one insect host (cutworms)	Poland	No	
E21-ENT-18	The influence of essential fatty acids and aphpa-tocopherol on the lipid metabolism and physiology of Colorado potato beetle and on the vitamin activity of alpha-tocopherol	Poland	No	
E21-ENT-19	Relationships in parallel development of insect host and parasites resistance to a common toxicant	Poland	Yes	18-C-17
E21-ENT-21 1/	One carbon metabolism in animals and in animal tissues cultured in vitro	Poland	No	
E30-ENT-1	Biological investigations of the pink bollworm (Pectinophora - Platyedra gossypiella Saund.) under the conditions of the southeast of the SR Macedonia	Yugoslavia	No	
E30-ENT-2	Investigations of leaf miners in Yugoslavia orchards	Yugoslavia	Yes	3-A-5
E30-ENT-3	Parasites, predators, and pathogenic organisms study of the cereal leaf beetle, (Oulema melanopa L.), and resistance of domestic and small grain varieties to the insect	Yugoslavia	Yes	7-D-2
E30-ENT-4 1/	Investigation of red spider mite (Panonychus/ Matatetranychus/ulmi) in orchards	Yugoslavia	No	
F4-ENT-2 2/	Survey of the insect fauna of Egypt	Egypt	Yes	17-A-9
F4-ENT-4	Biology, ecology, and utilization of insects other than honey bees in the pollination of agricultural crops	Egypt	Yes	15-F-4
F4-ENT-5	Studies on the insects attacking thistles in the U.A.R.	Egypt	Yes	18-B-6
F4-ENT-6	Studies of the control of houseflies and mosquitoes by means of chemosterilants in Egypt	Egypt	Yes	14-D-1,2
F4-ENT-9 1/	Sterile male release for control of the Mediterranean fruit fly	Egypt	No	
S3-ENT-1 2/	Biology and breeding of honey bees	Brazil	Yes	15-A-12
S3-ENT-7 2/	Catalogue of insects living on plants in Brazil and of the parasites and predators of the insects	Brazil	Yes	18-A-7
S5-ENT-2 2/	A biochemical study of Drosophila (vinegar flies) classification	Colombia	No	
S5-ENT-3 2/	The metabolism of temperature-accumulated Drosophila	Colombia	No	
S9-ENT-6 2/	Systematic collections, identification, and classification of the grasshoppers of Uruguay and neighboring territories of southern Brazil, southern Paraguay, and adjacent provinces of Argentina	Uruguay	No	
S9-ENT-7	Investigations on natural enemies of ants	Uruguay	Yes	14-C-2

1/ Initiated during reporting year.
2/ Terminated during reporting year.

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